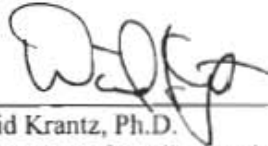


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Running head: HEART RATE VARIABILITY AND CATECHOLAMINE RESPONSES

HEART RATE VARIABILITY, CATECHOLAMINE AND HEMODYNAMIC RESPONSES
DURING REST AND STRESS IN CORONARY ARTERY DISEASE PATIENTS:
THE PIMI STUDY

by

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Abstract

Altered cardiac autonomic balance has been shown to be a predictor of adverse cardiac events in patients with cardiovascular disease (CAD). Alteration of the sympatho-vagal balance of heart regulation may be more evident during periods of mental stress. The high frequency (HF) component of heart rate variability (HRV) is a marker of the parasympathetic regulation of the heart. The meaning of the low frequency (LF) component of HRV is controversial, but it may represent the sympathetic modulation of heart regulation. Plasma catecholamine levels (epinephrine and norepinephrine) are markers of sympathetic nervous system action. Increases in hemodynamic (blood pressure and heart rate) responses to stress are predominantly regulated by the sympathetic branch of the autonomic nervous system (ANS). The relationships among the frequency domains of HRV, hemodynamics, and catecholamine level were examined at rest and during speech mental stress in 147 CAD patients from the Psychophysiological Investigations of Myocardial Ischemia (PIMI) study. At rest, there were no significant associations between HRV measures, hemodynamic responses, and plasma catecholamine levels. However, at peak stress levels, analyses of variance revealed a significant inverse relation between HF levels and epinephrine (Epi), (univariate $F_{(3, 132)} = 2.9; p = .037$); and a significant inverse relation between LF and catecholamine levels, (multivariate $F_{(6, 258)} = 3.7; p = .001$). At peak stress levels, HF levels were inversely related to systolic blood pressure (SBP) and heart rate (HR) ($F_{SBP(3, 141)} = 3.9; p = 0.01$; and $F_{HR(3, 141)} = 7.2; p < 0.001$). At peak stress levels LF was inversely related to SBP and HR, ($F_{SBP(3, 140)} = 8.9; p < 0.001$; and $F_{HR(3, 140)} = 4.0; p = 0.009$). These findings suggest that associations of the vagal components (HF) of HRV with sympathetic markers in CAD patients are only revealed under conditions of mental stress. In addition, based on the data, the LF component of HRV may not be exclusively sympathetic. An understanding of the

complex interplay between HR, HRV, BP and catecholamine level may allow for a more comprehensive evaluation the state of the sympatho-vagal balance during mental stress in CAD patients.

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Appendix A: Tables

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Introduction

Cardiovascular disease (CVD) is the number one killer of adults in the United States (Thom et al., 2006). Fifty three percent of all deaths due to CVD can be attributed to coronary heart disease (CHD) (CDC/NCHS, 2002). Myocardial infarction (MI) and sudden cardiac death (SCD) are major contributors to unexpected and premature death from CHD for both men and women. Over 12.5 million of the estimated 32 million worldwide MIs are fatal, and 40-75% of the victims died before reaching the hospital (WHO, 2002). It was estimated that in 2006 700,000 Americans would have a new coronary attack and about 500,000 will have a recurrent attack. It is also estimated that an additional 175 000 silent first heart attacks occur each year. In addition, about 335,000 people a year die of coronary heart disease. The majority of these are sudden deaths, which may be caused by a lethal arrhythmia leading to cardiac arrest (Malliani & Montano, 2004).

Coronary heart disease may lead to myocardial ischemia (inadequate blood supply to the heart muscle), MI, malignant arrhythmias and subsequent cardiac arrest (Krantz, Kop, Santiago, & Gottdiener, 1996; Krantz et al., 1999; Lampert et al., 2002). Research has shown that the occurrence of fatal arrhythmias and cardiac ischemia can be linked to both physical and mental stress (Alpert, Thygesen, Antman, & Bassand, 2000; Malliani & Montano, 2004; Mittleman et al., 1993). Acute mental stress leads to the increase of sympathetic and decrease of parasympathetic influences on the heart ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of

Pacing and Electrophysiology," 1996). The present study investigates the relationships among various markers of the autonomic functioning of the heart (heart rate variability and catecholamine levels) at rest and during mental stress in patients with coronary artery disease.

Myocardial Infarction (MI) and Sudden Cardiac Death (SCD)

Myocardial Infarction

Myocardial infarction (MI) is the rapid development of cardiac muscle (myocardium) necrosis. MI is a leading cause of death from CHD for men and women and is responsible for more than 221,000 deaths per year in the United States (Thom et al., 2006). One third of patients who experience acute ischemic heart disease die within 24 hours of the onset of the ischemia (defects in perfusion) and half of them die before reaching the hospital (Califf & Newby, 1996). In addition, almost half of MIs are silent, meaning that patients do not experience the classic symptoms of chest discomfort, shortness of breath, and diaphoresis (cold sweat) during acute ischemia (Antman & Fox, 2000).

Risk factors for atherosclerotic plaque formation may be divided into non-modifiable and modifiable. Non-modifiable risk factors are sex (male), age, and family history of premature CHD (Thom et al., 2006). The modifiable risk factors are smoking (Barth, Critchley, & Bengel, 2006; Kupersmith, Holmes-Rovner, Hogan, Rovner, & Gardiner, 1995), sedentary lifestyle and lack of exercise (Myers et al., 2002), poorly

managed hypertension, diabetes mellitus, dyslipidemia (McEwen, 2004), obesity (Schlundt, Hill, Sbrocco, Pope-Cordle, & Kasser, 1990), and psychosocial stress (Krantz et al., 1999).

Sudden Cardiac Death

Sudden cardiac death (SCD) is unexpected death resulting from cardiac arrest (sudden stop of the heart) within 1 hour of the onset of symptoms (Brugada & Andries, 1992; Rodriguez et al., 1992). Although more than 80% of SCD events occur in individuals with CHD, SCD is often the first expression (D. Muller, Agrawal, & Arntz, 2006). Approximately 300,000 deaths per year are attributable to SCD. Research suggests that impaired left ventricular dysfunction and frequent ventricular arrhythmias, such as ventricular tachycardia (VT) followed by ventricular fibrillation (VF), are the leading causes of SCD (Brugada & Andries, 1992; Brugada et al., 1991; D. Muller et al., 2006). The research also shows that VT is more common for patients with IHD due to the phenomenon of re-entry. Post-MI patients are at the highest risk for SCD in the first 6-24 months after infarction (Brugada & Andries, 1992; Brugada et al., 1991; D. Muller et al., 2006), but increased risk remains indefinitely (Moss, Daubert, & Zareba, 2002; Wilber et al., 2004). In addition, postmortem studies of SCD patients indicate that atherosclerosis and consequent ischemia were the most common causes of death (Burke et al., 1997).

The most common risk factors for SCD are a family history of SCD, prior-MI, and an ejection fraction of less than 30-35%. In addition, dysregulation of the autonomic control of the heart, including decreased vagal activity or/and increased sympathetic tone, has been linked to an increased susceptibility to lethal arrhythmias (Bruce et al.,

1977; Brugada et al., 1991; Brugada, Talajic, Smeets, Mulleneers, & Wellens, 1989). Zareba and Moss proposed the “lethal triad”, a complex interplay of numerous factors resulting in SCD (Zareba & Moss, 2003) (See figure 1). This triad includes dysregulation of the autonomic control of the heart; myocardial substrate (wall motion abnormalities, reduced ejection fraction, atrial fibrillation, etc); and myocardial vulnerability (ischemia, ventricular arrhythmias, T wave alternans, etc).

Mental Stress and Coronary Artery Disease (CAD)

Mental Stress and Stress response

Stress is a process by which environmental events (stressors) challenge or threaten the organism. The definitions of stress include external stressors (e.g., financial difficulties, planning a wedding, etc), stress responses (physical, psychological, emotional, and behavioral responses of the organism) or the consequences of stress (e.g., impaired immune system function, atherosclerosis, depression, sudden cardiac death, etc) (J. R. Kaplan, Pettersson, Manuck, & Olsson, 1991; M. S. Kaplan, Pratley, & Hawkins, 1991; Lucini, Mela, Malliani, & Pagani, 2002; Rozanski, Blumenthal, Davidson, Saab, & Kubzansky, 2005).

Stress is a state of threat to the organism’s homeostasis (Cannon, 1914). Adaptation to stress confers a survival advantage (McEwen, 2002). The autonomic nervous system, the hypothalamic–pituitary–adrenocortical (HPA) axis, and the cardiovascular, metabolic, and immune systems protect the body by responding to internal and external stressors and assuring successful adaptation to the challenge (McEwen, 1998; Selye, 1975a). Consequently, effects of stress or stress responses

involve the sympathetic nervous system (SNS) and HPA axis (Cannon, 1914; Selye, 1954; McEwen, 1998).

Stressful environmental stimuli trigger the release of corticotrophin releasing factor (CRF) which causes the release of adrenocorticotrophic hormone (ACTH) and cortisol into the blood stream (Frankenhaeuser & Rissler, 1970; Mason, Mangan, Brady, Conrad, & Rioch, 1961). The increased secretion of cortisol helps mobilize stored energy in response to perturbation, therefore increasing the individual's chance for surviving or enduring the physical challenge (McEwen, 1998).

In addition, the adrenal medulla receives messages from the brain through sympathetic nerves. Sympathetic activation constitutes the primitive “fight-or-flight” response that prepares the organism for the intense exertion necessary for survival in the face of threat (Selye, 1954). In response to stimulation of the sympathetic nervous system (SNS), the adrenal medulla releases epinephrine and norepinephrine (Frankenhaeuser, Mellis, Rissler, Bjorkvall, & Patkai, 1968; Howley, 1976). The physiological responses to catecholamine level include increases in blood pressure, peripheral resistance, HR and cardiac output (Guyton & Hall, 2000). SNS activation is balanced by the activation of the parasympathetic branch (PNS) of autonomic nervous system. However, chronic activation of the SNS may shift the autonomic balance towards the sympathetic system (McEwen, 2004).

Successful adaptation to stress depends on both the ability to respond to stress and the ability to control the response to stress. For example, predictability and controllability (or the perception of predictability or controllability) can reduce SNS and

HPA axis activation during a stress event (Frankenhaeuser, 1978). The evaluation of the stressful situation or event by the individual in terms of harm and resources to cope (appraisal) can also modify SNS and HPA axis responses to stress (Lazarus, 1984, 1992; Mason, 1968a, 1968b).

This activation of the SNS and HPA prepares the organism for exertion (vigorous motor activity). In today's society, however, stress is more likely to be psychological, not physical (Glass & Singer, 1972). The stress response to a psychological challenge is similar to the response to a physical challenge (i.e. increases in ACTH and cortisol secretion, heart rate, and blood pressure). However, psychological stress is different. It is not tied to an increased metabolic demand, and it does not have clear beginning or end (Krantz & McCeney, 2002; McEwen, 2004). Frequent activation of the HPA axis with associated increases in cortisol and sympathetic activation in response to psychological stressors has been linked to visceral obesity, hypertension, hyperlipidemia, and insulin resistance (Hautanen & Adlercreutz, 1993; Rosmond & Bjorntorp, 1998; Rosmond, Dallman, & Bjorntorp, 1998) and to an increased risk for chronic conditions such as cardiovascular disease, diabetes, and atherosclerosis (Bjorntorp, 1997; Karasek et al., 1988; McEwen, 2004).

Types of psychosocial stress: chronic, episodic and acute

The current research is focused on laboratory-induced acute mental stress and the responses of various markers of the autonomic regulation of the heart. It is believed that individuals with chronic or episodic stress are at higher risk for subsequent coronary events in the face of the acute challenge (acute stress) (Krantz & McCeney, 2002; J. E. Muller & Tofler, 1992; Suchday, Krantz, & Gottdiener, 2005).

Chronic stress is a state of ongoing physiological and psychological arousal of the organism (McEwen, 2002; J. E. Muller & Tofler, 1992). Examples of chronic stress are occupational stress (low control and high demand environment) (Karasek et al., 1988) and marital strain (Gjerdingen, McGovern, Bekker, Lundberg, & Willemssen, 2000). Acute mental stress results from exposure to short-term stressors such as terrorist attack, anger, public speaking, etc (Krantz & McCeney, 2002; Krantz et al., 1999; Leor & Kloner, 1996; McEwen, 2004). Finally, episodic mental stress is neither long term nor short term and ranges in duration from several months to a year (e.g. depression) (Carney et al., 2000; Kop, 1997).

Stress Reactivity

Definition and Measures

In the face of a challenge, the organism reacts with emotional, behavioral and physiological responses, or reactivity (Krantz & Manuck, 1984; Soufer, Arrighi, & Burg, 2002). Therefore, stress reactivity is the change in cardiovascular parameters (BP, HR, and cardiac output), sympathetic nervous system activity, and/or neuroendocrine/hypothalamic-pituitary-adrenal axis (Mason, 1968a, 1968b; Soufer et al., 2002) in response to a stressor.

Cardiovascular changes in response to real-life or laboratory-induced psychological challenges may be a marker of CHD risk (Krantz & Manuck, 1984). The cardiovascular system constantly changes in response to diverse activities, ranging from quiet rest to maximal exercise, performed by the organism. Therefore, the cardiovascular system is continuously "reactive," depending on the metabolic needs of the organism (Manuck, Kaplan, Adams, & Clarkson, 1989). It has been hypothesized that individuals

with exaggerated cardiovascular responses during stress may be more at risk for the development of cardiovascular syndromes such as hypertension or coronary heart disease than those exhibiting relatively small responses (Krantz & Manuck, 1984; Krantz & McCeney, 2002).

Cardiovascular reactivity to mental stress can be measured by using a number of different parameters. Most frequently cardiovascular reactivity is determined by examining the differences between conditions of rest and stress in heart rate, systolic and diastolic blood pressures (Kamarck, Jennings, Pogue-Geile, & Manuck, 1994). Individuals differ in terms of their inclination to exaggerated cardiovascular responses during laboratory mental stress. This exaggeration may be cardiac (an increase in cardiac output, HR, etc) or vascular (an increase in peripheral resistance and blood pressure) (Dembroski & MacDougall, 1983).

Research has shown that both types of exaggerated cardiovascular responses during mental challenges are relatively stable over time (Kamarck et al., 1994). This implies a consistency in the response by the individual when confronted by stress at other points of time. Furthermore, individuals who exacerbate the exaggerated cardiovascular responses during laboratory mental stress have an exaggerated cardiovascular response during daily life in response to mental stress (Allen et al., 1987; Manuck & Garland, 1980; Suchday et al., 2005).

Pathophysiology of exaggerated responses to mental stress

When the brain perceives acute mental stress as a challenge or increased demand on the body, it activates the sympathetic nervous system and inactivates the parasympathetic influences in order to prepare the organism for exertion (Mason, 1968a; McEwen, 2004; Selye, 1975c). This activation includes, through impact on beta-adrenergic receptors, an increased contractility of the myocardium and an increase in heart rate, followed by an increase in cardiac output, and finally an increase in vascular resistance (small artery constriction) (Strike et al., 2004). All of these result in increased systolic and diastolic blood pressures. Myocardial oxygen consumption is determined by heart rate, contractility, and wall stress (which is determined by systolic pressure and chamber radius). By increasing all three major determinants, sympathetic stimulation increases myocardial oxygen consumption significantly (Guyton & Hall, 2000). Thus, in the face of the acute stress, cardiac demand on the oxygen supply increases (Sherwood & Turner, 1995; Soufer, 2004).

Furthermore, the sympathetic responses of the heart to stress are accompanied by a decrement in vagal tone (Soufer, 2004). Vagal withdrawal during stress and a shift of the autonomic balance towards sympathetic influence leads to an increase of myocardial oxygen consumption, hemodynamic resistance, myocardial contractility, and a decrease in arrhythmic threshold of the heart. These changes in cardiovascular functioning may lead to fatal arrhythmias and MIs (Krantz et al., 1996; J. E. Muller, Tofler, & Edelman, 1989). For example, the recall and description of an anger-provoking event may induce left ventricular dysfunction and coronary vasoconstriction (Krantz et al., 1999). Severe stressors, such as earthquakes and terrorist attacks, are also associated with an increased risk of SCD (Kark, Goldman, & Epstein, 1995). For example, Leor and colleagues

(1996) reviewed coroner records in the state of California, and found an increase in the number of SCDs and acute MIs during the week after the Northridge earthquake in comparison to the week before earthquake (Kark et al., 1995; Leor & Kloner, 1996; Leor, Poole, & Kloner, 1996).

Research shows that humans and animals with exaggerated cardiovascular responses to mental challenges are at a higher risk of subsequent coronary events and CHD compared to those with normal cardiovascular responses to mental challenge. For example, Manuck and colleagues (1997) found that cynomolgus monkeys who exhibited heightened cardiovascular reactions to psychological stress had higher levels of atherosclerosis in comparison to monkeys with normal stress reactivity. Matthews et al. (2006) found that exaggerated cardiovascular response (SBP response) of young adults (human) while playing a video game predicted increased coronary calcification 13 years later. In another study, individuals at high risk for essential hypertension exhibited higher cardiovascular reactivity (HR, BP responses) during a public speaking stressor task than did individuals with a lower risk for essential hypertension (al'Absi & Wittmers, 2003).

Stress reactivity in CHD patients

The relationship between exaggerated cardiovascular responses to acute stress and subsequent adverse cardiac events may be more evident in patients with clinical or sub-clinical CHD. For instance, individuals with preclinical CHD who had exaggerated cardiovascular responses to mental stress exhibited higher levels of cardiac ischemia during exercise in comparison to individuals with normal levels of stress reactivity (Kral et al., 1997). Post-MI patients with exaggerated cardiovascular reactivity to mental stress

were at a higher risk for development of new clinical events (re-infarction or stroke) during the 39 to 64 months after acute MI in comparison to post-MI patients with normal cardiovascular reactivity (Manuck, Olsson, Hjendahl, & Rehnqvist, 1992). In another study, the relationships between hemodynamic reactivity and myocardial ischemia during laboratory mental stress were evaluated in 39 CAD patients and 12 controls (Krantz et al., 1991). Results revealed that SBP levels during mental task and SBP reactivity (increases) were highest for the severely ischemic group and lowest for controls (Krantz et al., 1991). Acute, laboratory-induced anger as part of a mental stress task has also been found to increase the risk of cardiac arrhythmias associated with sudden cardiac death in CAD patients with implantable cardioverter-defibrillators (Kop et al., 2004; Lampert, Jain, Burg, Batsford, & McPherson, 2000).

In summary, research has shown a link between acute mental stress and subsequent coronary events (e.g., MI or SCD). This relationship may be attributed to activation of the sympathetic nervous system and deactivation of the parasympathetic nervous system leading to increased myocardial demand. The increased demand on the myocardium in patients with known or sub-clinical CHD can be detrimental and may lead to an increase in myocardial ischemia (including MIs) and lethal arrhythmias.

Autonomic Nervous System

Role of Autonomic Nervous System

The autonomic nervous system helps to control arterial pressure, gastrointestinal secretion, and body temperature among other things. Essentially all of the organs and

systems of the body perform functions that help to maintain a homeostasis for the organism in the environment (Guyton & Hall, 2001; McEwen, 2004). This homeostasis is crucial for the survival of the organism (Cannon, 1914). The autonomic nervous system, which is activated by centers located in spinal cord, brain stem and hypothalamus, is one of the most powerful systems in maintaining this constant state. The ANS includes the sympathetic and parasympathetic nervous systems. Sympathetic stimulation causes excitation effects in some organs (e.g., arterioles) and inhibitory effects in others (e.g., bladder). Parasympathetic stimulation also displays selective excitation and inhibition (Guyton & Hall, 2000). Most of the organs are dominantly controlled by one or another branch of the ANS. However, in the heart, bronchi, and liver the two systems act reciprocally (Branding, 1998).

The parasympathetic nervous system tends to be highly specific to an organ or system in its activation, while the sympathetic nervous system is capable of producing a mass discharge of almost all its portions (McEwen, 2002; Selye, 1975a). This mass discharge is called an alarm reaction or stress response (Mason et al., 1961; Selye, 1975b). This frequently occurs when the hypothalamus is activated by psychological stress (e.g., fright, anger) or physical stress (e.g., injury) (Mason, 1968a, 1968b). This mass discharge directly increases the ability of the body to perform vigorous muscle activity by enhancing the work of certain organs and systems and inhibiting the others. For example, the discharge increases mental concentration, heart contractility, heart rate, blood pressure, and decreases the work of the organs that are not needed for motor activity (e.g., kidneys) (Guyton & Hall, 2000).

Autonomic Regulation of the heart

The heart is supplied with both sympathetic and parasympathetic nerves. The parasympathetic nerves stem from the vagal nerve and are distributed to the sino-atrial (S-A, the pacemaker) and atrio-ventricular (A-V) nodes of the conduction system of the heart (Zipes, Barber, Takahashi, & Gilmour, 1983). There are also vagal efferents to the atrial and ventricular myocardium, although these are significantly less numerous compared to nodal tissue (Zipes et al., 1983). Stimulation of the parasympathetic nerves to the heart causes the neurotransmitter, acetylcholine, to be released from nerve endings. Acetylcholine slows the transmission of the impulses to the A-V node, which decreases HR and slightly decreases the strength of the heart muscle contraction (the rate of heart pumping) (Guyton & Hall, 2000). This, in return, will decrease cardiac output, and also decrease the cardiac demand for the oxygen. Under resting conditions, the parasympathetic influence (vagal tone) dominates that of sympathetic influence on the heart (Bernardi, Saviolo, & Spodick, 1989).

The sympathetic nerves are distributed to all parts of the heart, with increased concentration in the ventricles. Stimulation of these sympathetic fibers causes release of norepinephrine from the nerve endings (direct effect on the heart). In addition, the overall activation of the sympathetic nervous system causes a discharge of epinephrine and norepinephrine into the blood stream from the adrenal medulla (indirect effect, slower) (Guyton & Hall, 2000). Norepinephrine and epinephrine increase heart rate and the force of myocardial contraction (Bernardi et al., 1989), which increases ejection fraction and the volume of the blood pumped by the heart, possibly resulting in an increased demand by the heart muscle for oxygen (Guyton & Hall, 2000).

Therefore, the parasympathetic nervous system produces direct inhibitory effects on the heart and sympathetic nervous system produces direct and indirect excitatory effects on the heart muscle.

Catecholamines and CHD

Catecholamines, including norepinephrine and epinephrine, are neurohumoral messengers of the sympathetic nervous system. In contrast to the parasympathetic nervous system, which has only direct effects, the sympathetic nervous system has both direct and indirect effects on the organs and systems of the body (Mason, 1968a, 1968b; Mason et al., 1961). The direct effect of the sympathetic nervous system is accomplished through stimulation of the sympathetic nervous fibers and release of norepinephrine by the terminal nerve endings onto receptors of the effector organs (a neural effect). The indirect effects are accomplished through stimulation of the sympathetic fibers of the adrenal medulla. This causes large quantities of Epi and NE to be released into the circulating blood flow. Circulating epinephrine and norepinephrine have almost the same effects on organs and systems as the effects caused by direct sympathetic stimulation, except that the effects of blood catecholamine levels last 5-10 times longer, and there is a delay of effects because of the increased time needed to reach effector organs (Mason, 1968a, 1968b; Mason et al., 1961).

Activation of the sympathetic nervous system causes the excitation of the heart muscle through increases of contractility and heart rate. This may lead to an increased demand for oxygen by the heart and myocardial ischemia (Ramachandruni et al., 2006). Sympathetic activation may also decrease the threshold for arrhythmic vulnerability (Lampert et al., 2002; Lampert et al., 2005). These relationships may be more

pronounced in patients with known CHD. An alteration of the autonomic control of the heart has been demonstrated to be both a possible cause and a consequence of an ischemic episode (Mainardi, Bianchi, & Cerutti, 2002). An increase in sympathetic activity was observed after coronary occlusions in humans and animals (Joho et al., 1999; Rimoldi et al., 1990). Moreover, changes or alterations of autonomic tone on the heart have been observed as triggers of spontaneous ischemia (Chierchia, 1997; Joho et al., 1999; Lanza et al., 1996). Ramachndruni et al. (2006) found that laboratory mental stress induced ischemia (defects of perfusion) in CHD patients. Laboratory mental-stress has also been demonstrated to increase the levels of arrhythmic vulnerability in ICD patients (Lampert et al., 2000; Lampert et al., 2005).

In summary, the sympathetic and parasympathetic nervous systems provide reciprocal autonomic regulation to the heart. Under resting conditions parasympathetic tone prevails over sympathetic tone on the heart. The sympathetic nervous system, through nerve fibers and blood catecholamine levels, produces direct and indirect excitation of the heart muscle. The mass discharge of the sympathetic nervous system in response to psychological and physical stress increases cardiac oxygen demand and may decrease the threshold for arrhythmic vulnerability. Evidence supports that patients with CHD may have alteration of the autonomic control of the heart. These coupled with effects of the acute stress in patients with CHD may lead to negative outcomes such as SCD or MI.

Blood pressure

The function of the circulation is to transport nutrients and hormones to the appropriate tissues and transport waste products away in order to maintain an appropriate environment in all the tissues (Guyton & Hall, 2000). This constant flow of blood is assured through appropriate blood pressure (BP). Blood pressure (arterial pressure) is defined as force exerted by the blood against any unit area of the vessel wall (Guyton & Hall, 2000). Arterial pressure fluctuates between systolic blood pressure level (mean = 120 mm Hg) and diastolic blood pressure level (mean = 80 mm Hg). Blood pressure levels depend on systemic vascular resistance and cardiac output (Guyton & Hall). The sympathetic nervous system carries mostly vasoconstrictor fibers and only a few vasodilator fibers into the systemic vessels. Under normal conditions, all vessels are under continuous moderate sympathetic stimulation (vasomotor tone) (Guyton & Hall, 2000).

The regulation of blood pressure is very complex. Regulation of BP is traditionally described in terms of homeostasis. Blood pressure is continuously perturbed by external stimulations, but it displays the tendency to come back toward a reference point. In addition, the autonomic nervous system is capable of causing a rapid increase in blood pressure (Guyton & Hall, 2000). For this purpose, the entire vasoconstriction and cardioaccelerator functions of the sympathetic nervous system are stimulated as a unit. There is also reciprocal inhibition of vagal signals to the heart (parasympathetic inhibition (Guyton & Hall, 2000) resulting in an increased vascular resistance, thereby increasing arterial pressure, and an increased volume of blood in heart chambers, thereby increasing cardiac output and blood pressure. Direct sympathetic stimulation of the heart increases heart rate and contractility; thus increasing cardiac output and thereby

blood pressure (Guyton & Hall, 2000). These acute increases in blood pressure resulting from the mass activation of the sympathetic nervous system can be triggered by acute mental stress (Krantz & Manuck, 1984; Reims et al., 2004). Furthermore, individuals who have higher increase in blood pressure from rest to stress (blood pressure reactivity) are at higher risk of the development of CHD (Armario et al., 2003; Eliasson, Hjendahl, & Kahan, 1983; Sherwood, Hinderliter, & Light, 1995; Sherwood & Turner, 1995). Some research indicates that blood pressure reactivity in response to laboratory induced acute mental stress has a stronger association with future cardiovascular damage and hypertension than do other reactivity measures (e.g., heart rate; forearm blood flow; vascular resistance) (Krantz et al., 1999; Manuck et al., 1992; Nazzaro et al., 2005).

In summary, blood pressure is predominantly regulated by direct and indirect sympathetic stimulation. The activation of the sympathetic system corresponds with an acute increase in blood pressure. In addition, individuals who are more reactive in terms of the blood pressure increase in response to mental stress are at higher risk of subsequent CHD.

Heart Rate Variability

Heart Rate Variability (HRV) or Respiratory Sinus Arrhythmia (RSA)

The heart period, or time between successive normal beats, is based on two components: the intrinsic firing rate of the SA node of the heart, and the collective input of sympathetic and parasympathetic nervous systems (McMillan, 2002). At rest, the rhythm of the heart is primarily under the control of the vagus nerve, which inhibits

heart rate and the force of contraction (Bernardi et al., 1989). During inhalation, vagal nerve activity is impeded and the heart rate increases due to a drop in intrathoracic pressure and consequent fall in systolic blood pressure. The change in blood pressure is sensed by the baroreceptor of the carotid sinus (Bernardi et al., 1989; Appel et al., 1989). When we exhale, the pattern is reversed (Appel, Berger, Saul, Smith, & Cohen, 1989). Therefore, the intervals between normal successive beats are not equal, and represent rhythmic activation and inactivation of sympathetic and parasympathetic control of the heart. This rhythmic fluctuation of the heartbeat is called respiratory sinus arrhythmia (Bernardi et al., 1989). The magnitude of the changes in heart rate in response to changes in blood pressure reflects the sensitivity of the baroreceptor and the integrity of the parasympathetic nervous system.

There are other sources of HRV including circadian changes, standing up, head tilt, exercise, and psychological stress. The greatest variation of the heart rate occurs with circadian changes (difference of the HR between night and day) ("Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996; Kleiger, Stein, & Bigger, 2005). These changes are mediated by complex but poorly understood neurohormonal mechanisms (Kleiger et al., 2005). In general, after waking up, the markers of sympathetic activity (HR, BP, catecholamine level) have been shown to rise rapidly and concomitantly with a simultaneous vagal withdrawal (Furlan et al., 1990). This corresponds to a decrease in HRV because of vagal withdrawal. Therefore, the rhythm of the heart is also affected by circadian patterns of sympatho-vagal balance. In addition, standing up or tilting the head

upright corresponds to a decrease of HRV, because of vagal withdrawal and minimal sympathetic activation of heart control (Bloomfield et al., 1997; Bloomfield et al., 2001).

The sympathetic activation and parasympathetic inactivation of heart control during alarm reaction (stress response) (Carney et al., 2000; Kleiger et al., 2005) also corresponds to a decrease in HRV. Pagani et al. (1991) found that psychological challenges enhance sympathetic activity in dogs therefore decreasing HRV.

In summary, HRV represents a rhythmic fluctuation of the heart rhythm. These fluctuations are evident at rest, standing up, at awakening, and during mental and physical challenges, and represent the autonomic balance of the cardiac control by both branches of the ANS.

Measures of HRV

The techniques of measuring heart rate variability can be divided into two categories: frequency domain and time domain methods. Time domain measures are the simplest to perform. Using this method, the heart rate at any point in time or the intervals between successive normal complexes are determined (Malik & Camm, 1993). Simple time domain measures can be calculated using the mean period between normal beats (NN intervals), the mean HR, standard deviations of the NN intervals (SDNN), the differences between the longest and shortest NN intervals, and the differences between nocturnal and diurnal heart rate (Task Force, 1996).

Frequency domain measures are used to calculate HRV in both short-term (2-5 minutes) and long term recordings (24 hours) (Akselrod et al., 1981). However, traditionally, spectral analyses are done in laboratory studies for short term recordings of HRV (Stein, Bosner, Kleiger, & Conger, 1994; Stein, Domitrovich, Huikuri, & Kleiger,

2005). There are various spectral methods used to calculate the frequency domain of the HRV. Power spectral density (PSD) indicates how power distributes as function of frequency (Task Force, 1996; Akselrod et al., 1981). Three main spectral components of HRV are high frequency power (HF, 0.15-0.40 Hz), low frequency power (LF, 0.04-0.15 Hz), and very low frequency power (VLF \approx 0) (Luczak & Laurig, 1973). Research has shown that HF reflects the vagal (parasympathetic) control of the heart, more specifically RSA (Bloomfield et al., 2001; Brown, Gee, Olah, Docker, & Taylor, 1992; Luczak & Laurig, 1973; Stein et al., 1994; Stein et al., 2005).

LF power is modulated by baroreflexes and a combination of sympathetic and parasympathetic efferent impulses on the SA node (Billman, 1986; Bloomfield et al., 1997; Kleiger et al., 2005; Stein et al., 1994). Therefore, LF represents both sympathetic and parasympathetic control of the heart.

However, some researchers indicate that LF and changes in LF predominantly represent the sympathetic regulation or sympathetic activation of the heart (Malliani & Pagani, 1991; Malliani, Pagani, Lombardi, & Cerutti, 1991; Montano et al., 1996; Pagani, Rimoldi et al., 1991; Piccirillo et al., 2006). Pagani et al., (1991) investigated changes in LF power and systolic blood pressure (SBP) variability in men in response to mental arithmetic and in conscious dogs in response to mental stress. In both cases LF of HRV and SBP variability increased significantly suggesting enhanced sympathetic activity. Montano et al. (1996) found positive correlations between the discharge of the sympathetic neurons responsible for the cardiac control in the brain and increases in the LF component of the HRV and arterial blood pressure in cats. Furthermore, Lombardi et al. (1994) and Montano et al. (1994) have suggested that evaluation of the LF in

normalized units can help to assess the state of sympathetic modulation of the heart (Malliani, Lombardi, & Pagani, 1994; Montano et al., 1994).

Even though various mathematical manipulations of LF power have been used to better assess the sympathetic control of the heart (normalization of LF power, use of the ratio LF/HF; Kleiger et al., 2005), they have produced mixed results. In some of the cases, the increase in LF/HF was misinterpreted as an increase of sympathetic activity when the changes were actually due to vagal withdrawal and subsequent decrease in HF power (Kleiger, 2005; Task Force, 1996). Koh et al. (1994) showed that LF variability in patients with interrupted outflow of the sympathetic output was comparable to normal subjects. However, injection of atropine (a parasympathetic inhibitor) completely disrupted LF rhythm (Koh, Brown, Beightol, Ha, & Eckberg, 1994). Other studies have showed positive correlations between LF and SBP variability in heart transplant patients (Constant et al., 1995; Hughson, Maillet, Dureau, Yamamoto, & Gharib, 1995).

In summary, the frequency domain of HRV is usually used for short term recordings. HRV variability and its components reflect the sympatho-vagal regulation of the heart by the ANS. Research indicates that HF represents the vagal activity on the heart muscle. In the case of LF, research is less conclusive. Some researchers believe that LF indexes the sympathetic regulation of the heart, and others believe that changes in LF are due to both changes in parasympathetic sympathetic controls on the heart.

HRV and CHD

HRV reflects the autonomic control of the heart. Reduced HRV is thought to reflect an imbalance of the autonomic regulation of the heart and can serve as a risk factor or predictor of future adverse cardiac events (e.g. MI and SCD) (Carney et al., 2000; Lampert et al., 2002; Lewis, 2005; Mainardi et al., 2002). Such an imbalance is hypothesized to be caused by reduced parasympathetic tone on the SA node and/or an increase in sympathetic firing (Lewis, 2005; Lombardi, Malliani, Pagani, & Cerutti, 1996; Stein & Reddy, 2005). This imbalance is especially heightened in CHD patients. For example, the less variable the heart signals are the greater the risk of post-MI mortality from re-infarction or fatal arrhythmias due to ventricular tachycardia (McMillan et al., 2002; Task Force, 1996). Wolff et al. (1978) observed two groups of patients ($n = 176$) after acute MI. Patients with reduced R-R intervals or reduced sinus arrhythmia ($n = 73$) had higher in-hospital mortality than patients with normal sinus arrhythmia ($n = 103$).

A series of studies examined the prediction of overall cardiac mortality in post-MI patients based on reduced HRV, decreased ejection fraction, and the frequency of ventricular ectopy (Farrell et al., 1991; Odemuyiwa et al., 1991). These studies found that the predictive value of reduced HRV was comparable to that of left ventricular ejection fraction. However, HRV is a better predictor than ejection fraction for arrhythmic events (SCD and ventricular tachycardia) in post-MI patients (Odemuyiwa et al., 1991). Pozzati et al. (1996) analyzed 24-hour ECG tapes of 8 patients with CHD who died suddenly and 24 tapes of the patients with CHD who did not have life-threatening arrhythmias. Patients who subsequently had SCD had a significant decrease in HRV 5

minutes before the lethal outcome, and the control group did not have such a marked decrease in HRV (Pozzati, Pancaldi, Di Pasquale, Pinelli, & Bugiardini, 1996). The decrease in total HRV power was followed with ST segment depression (myocardial ischemia) and then sudden cardiac death (Pozzati et. al., 1996). These data suggest that sympatho-vagal imbalance may trigger fatal arrhythmias during acute ischemia and result in SCD in CHD patients (Pozzati et al., 1996). Other studies found a reduction in HF power and an increase in LF and LF/HF at rest and especially during stress in post-MI patients (Kamath & Fallen, 1991; Lombardi & Malliani, 1992). These changes may indicate the shift of sympatho-vagal balance towards sympathetic influence (Task Force, 1996). In addition, the changes of the spectral profile of HRV in acute post-MI patients are similar to those observed in heart failure patients or heart transplant patients (Arora et al., 2004). The decreases in HF and/or increases in LF and LF/HF ratio in acute post-MI patients very likely reflect diminished responsiveness of the heart or SA node to parasympathetic stimulation (Malliani, Lombardi, & Pagani, 1994; Malliani, Lombardi, Pagani, & Cerutti, 1994) or persistently high sympathetic tone on SA node (Malik & Camm, 1993; Malliani & Montano, 2004).

It is important to mention that large studies such as the European Myocardial Infarction Amiodarone Trial (EMIAT, N = 743) and Autonomic Tone and Reflexes after Myocardial Infarction (ATRAMI, N = 1139) (did not have n-note to insert the references) found that HRV is predictive of future cardiac death in post-MI patients, however it was not predictive of future arrhythmic events. This discrepancy in findings may be attributed to the present definition of SCD. The present definition of SCD includes not only patients with arrhythmic events, but also patients with re-infarction

and other CVD (Domanski et al., 2003; Greene et al., 1989). Because not every SCD is an arrhythmic event, the superiority of HRV in predicting future arrhythmic events is questionable.

Mental Stress and HRV

The relationship between worsening CHD and HRV as a marker of distorted autonomic regulation of the heart is more evident under stress conditions (psychological or physical stress). Specifically, studies examining HRV parameters during mental stress demonstrated the autonomic imbalance of the heart regulation, which was not evident at rest alone (Gianaros et al., 2005; Ruediger, Seibt, Scheuch, Krause, & Alam, 2004). Postmenopausal females ($n = 96$) who had a higher reduction in HF in response to a mental stress task (3 min speech preparation) had more calcification in coronary arteries in comparison to females with less reduction in HF in response to a psychological stressor (Gianaros et al., 2005). This relationship remained significant after adjustment of other risk factors (age, smoking status, and hormone therapy). Ruediger et al., (2004) compared HRV at rest and during mental stress in 20 male hypertensive (HT) subjects and 20 normotensive (NT) male subjects. HF was significantly reduced in HT subjects from rest to stress in comparison to NT subjects. LF was also significantly higher in HT subjects, in comparison to NT subjects during a mental arithmetic task. Kop et al. (2001) studied 19 male patients with stable CHD immediately before and after transient ischemic events. The results showed that patients had a significant decrease in HRV up to one hour before an ischemic event (HF and LF) (Kop et al., 2001). In addition, all

ischemic events were asymptomatic (no angina) and the decreases in HRV (HF and LF) were correlated with high levels of mental activity.

In summary, changes in HRV are thought to reflect the autonomic regulation of the heart. In CHD patients, the sympatho-vagal balance of heart regulation tends to be shifted more towards sympathetic influence, which may be reflected in a decrease of HF power and/or an increase in LF and LF/HF. It is believed that this change in HRV parameters due to sympathetic shift may be considered as an indicator and a precipitant of adverse cardiac events (myocardial ischemia or/and ventricular arrhythmia) in CHD patients.

Previous Research in autonomic markers of heart regulation

HRV provides a noninvasive method for investigating the dynamic influences of sympathetic and parasympathetic ANS on cardiac regulation (Lewis, 2005). Specifically, the HF component of HRV has been proposed to be a good marker of the parasympathetic regulation of the heart. Catecholamines (Epi and NE) are neurohumoral markers of the sympathetic nervous system (Guyton et al., 1991). Sympathetic activation can trigger malignant arrhythmias, whereas parasympathetic activity may exert a protective effect during a challenge (Schwartz et al., 1992). Blood pressure regulation can be described in terms of homeostasis (Guyton, 1991). The sympathetic branch of the ANS is responsible for the increase of blood pressure (Guyton, 1991; Parati et al., 1995). Experimental observations provide new information about the relationship between the state of the sympatho-vagal balance and the likelihood of adverse cardiac events. For

example, reduced HRV or a depressed chronotropic reflex in response to BP rise in conscious post-MI dogs identified dogs at the greater risk of developing malignant arrhythmias during an ischemic episode (Pagani et al., 1991). Furthermore, research indicates that people, especially CHD patients, who have sympatho-vagal imbalance in the regulation of the heart, are at a higher risk for aversive cardiac events (MI, lethal arrhythmias) (Lucini et al., 2002; Stein et al., 2005; Stein & Reddy, 2005). This pathophysiological relationship is especially pronounced during mental stress (Lampert et al., 2005; Rozanski et al., 2005). The analyses of the autonomic control of the heart by using a range of markers (HRV, catecholamine level, and BP) may help to get better information about the state of sympatho-vagal balance of the heart. This information will enable researchers and clinicians to identify people at higher risk for future complications from CHD while intervention may still benefit the patient.

Various researchers have studied the relationships between various markers of ANS functioning during mental stress. Lane et al. (1992) evaluated cardiovascular responses to mental stress (arithmetic task) in 25 healthy male subjects and hypothesized that subjects with lower RSA or HF at rest will be more reactive during the mental stress task. Subjects who had low values of normalized RSA at rest had higher levels of blood pressure during rest

($r = -.31$) and during stress ($r = -.31$). However, the correlations between SBP change scores and resting RSA were not significant ($r = -.15$) (Lane, Adcock, & Burnett, 1992).

Reims et al. (2004) evaluated the relationships among plasma catecholamine level and SBP at rest and during mental stress in men ($n = 20$) with high blood pressure and in men ($n = 25$) with normal blood pressure. They have found that correlations

between BP changes and NE ranged from .30 to .33, and for Epi from .39 to .46. The correlation coefficients were higher for men with high BP, but they were also significant for men with normal BP.

Lampert et al. (2005) studied changes in BP, catecholamine level, HF, HR and T-wave alternans (TWA) from rest to mental stress (anger recall and mental arithmetic) in 33 ICD patients. In the 33 patients, significant changes from rest to stress in catecholamine level, BP, HF, TWA and HR ($p = .01 - .001$) were observed (Lampert et al., 2005). HF decreased and the rest of the variables increased from rest to stress. In addition, the TWA changes significantly correlated with changes in BP, Epi and HR. However the researchers did not investigate the relationships among the variables such as catecholamine level, HF, LF, and BP.

Study Rationale

The use of multiple indicators (HRV, BP, catecholamine level) of autonomic functioning may provide a comprehensive evaluation of the state of the sympatho-vagal balance of the heart. It is possible that the analysis of autonomic control of the heart using a range of indirect markers of ANS functioning may represent a new way for identifying cardiac patients at a higher risk for adverse cardiac events. Some disturbances in autonomic regulation of the heart functioning are more evident under acute stress conditions than at rest. In addition, the presence of the sympatho-vagal imbalance of the heart regulation is more common for the CHD patients and the presence of such imbalance under mental stress or any other challenge for the organism is more detrimental for cardiac patients.

There are no current studies which have investigated the relationships among markers of autonomic functioning (HRV, BP, and catecholamine level) at rest and during mental stress in CHD patients. Lane et al. (1992) examined the relationship between RSA (HF) and SBP at rest and during mental stress. However, the relationships among catecholamine level and SBP and HF were not investigated. Furthermore, the researchers studied healthy subjects and did not investigate the LF component of HRV. Reims et al., (2004) compared levels of catecholamine level and BP during mental stress in hypertensive and normotensive subjects. The relationship among HRV domains, catecholamine level and BP were not investigated. Lampert et al., (2005) examined the relationship of T-wave alternans with BP, HF, catecholamine level, and HR during mental stress in ICD patients but the researchers did not assess relationships among BP, HF, catecholamine level, and HR.

Therefore, the current study's objective is to examine the relationships among sympathetic (Epi, NE, BP, HR, LF) and parasympathetic (HF) markers of cardiac functioning in CHD patients at rest and during mental stress. Study hypotheses are as follows:

Hypothesis I

At rest there is relatively little sympathetic influence on the heart. Mental stress leads to a decrease in parasympathetic stimulation and an increase in sympathetic stimulation on the heart (including discharge of Epi and NE) in order to keep up with an increased demand of the heart muscle and other organs and systems. The impairment of the autonomic regulation of the heart (vagal withdrawal or/and over activity of the sympathetic regulation) is more evident during mental stress, because of the overall

activation of the sympathetic system. Therefore, it is hypothesized that: (a) that there will be no relationship between levels of HF and catecholamine at rest; and (b) CHD patients with lower levels of HF will have higher levels of catecholamine during mental stress.

Hypothesis II

The interpretation of the LF component of HRV is controversial. It may represent either sympathetic regulation of the heart or represent influences of both branches of the ANS, sympathetic and parasympathetic. Pagani et al., (1991) propose that LF predominantly reflects sympathetic modulations of the cardiac sympatho-vagal influences. As previously discussed, at rest, S-A is primarily under parasympathetic modulation. However, during mental stress, the sympatho-vagal balance of the cardiac autonomic regulation shifts toward the sympathetic branch because of the overall sympathetic discharge. Therefore, it is hypothesized that: (a) that there will be no relationship between levels of LF and catecholamine at rest; and (b) CHD patients with higher levels of LF will have higher levels of catecholamine during mental stress.

Hypothesis III

The sympathetic branch of the ANS is responsible for the regulation of blood pressure. During mental stress, hemodynamics (HR, SBP, and DBP) increase due to parasympathetic withdrawal and overall sympathetic discharge. In order to investigate the relationships among hemodynamics (sympathetic influences), HF (parasympathetic), LF (sympathetic), and catecholamine (sympathetic) it was hypothesized that: (a) at rest there will no relationships between HF (LF) and SBP and DBP and HR; and (b) during mental stress, patients with lower levels of HF (during mental stress) will have higher

levels of HR, SBP, and DBP in comparison to patients with high levels of HF. During mental stress patients with higher levels of LF will have higher levels of HR, SBP, and DBP in comparison to patients with lower levels of LF.

Methods

In order to test the proposed hypotheses, the limited access dataset Psychophysiological Investigations of Myocardial Ischemia (PIMI) was requested from National Institute of Health (NIH). The data were released by the NIH in March 2002 for analyses in the present study.

Participants

Procedures for the PIMI study have been described elsewhere (Kaufmann et al., 1998). Briefly, 196 patients with stable CAD participated in at least one clinic visit during the investigation. Participants were recruited from four clinical units in the asymptomatic cardiac ischemia pilot study (ACIP). Sixty-four of these participants previously participated or were screened for participation in ACIP (Tamesis et al., 1993). The other participants were recruited from catheterization, exercise laboratories, or chart review. The primary goal of PIMI was to test the hypothesis that manifestation and expressions of cardiac ischemia are influenced by specific psychophysiological mechanisms.

Eligibility was established during a qualifying visit, at which time a standard exercise treadmill test was administered. Patients were eligible for the participation in

PIMI if they had all of the following: 1) CAD verified by angiogram, showing narrowing in diameter of at least one major coronary artery by $\geq 50\%$, or verified myocardial infarction; 2) evidence of myocardial ischemia on an exercise treadmill test, while patient was off any anti-ischemic medications, indicated by ST-segment depression ≥ 0.1 mV and confirmed by reading at the rest and exercise ECG Core Laboratory; 3) a completed 48-hour ambulatory electro cardiogram (AECG) while off medications; and 4) consent to participate.

Patients were excluded for any of the following: pregnancy, MI within 3 months of qualifying exercise tolerance test (ETT), percutaneous transluminal coronary angioplasty (PTCA) within 6 months of qualifying ETT, cardiac surgery requiring thoracotomy, unstable angina within 4 weeks of qualifying ETT, serious non-cardiac illness (e.g., renal, pulmonary), or an inability to discontinue medication.

Study Procedure

The study protocol included 3 visits: Qualifying Visit, Day 1 Visit, and Day 2 Visit. The purpose of the Qualifying Visit was to determine medical eligibility for the participation which included ETT and AECG in order to determine ischemia during daily life. Study Visit 1 occurred within 3 months of the qualifying visit and consisted either of a mental stress day, or a physical stress day. Visit 2 occurred 2 weeks after Visit 1 and consisted of either a mental stress day or a physical stress day (which ever was not previously assigned). In order to detect the effects of sequence of the mental and physical stress days, and the sequence of mental stress tests the order of the sequences were randomly determined for each patient at Visit 1. (See figure 1.)

After signing informed consent, all patients were asked to discontinue the anti-ischemic and other medications for all study visits. For more details about the type of medications and duration of the discontinuation see Kaufman and colleagues (1998).

Mental and Physical Stress

The mental stress day included two 5-minute mental stress tests: the Stroop and speech tasks. The sequence of the two mental stress tests was randomly determined for each patient. Instructions were provided from a standard script read by staff. For the purpose of the present study, only data collected during speech task were used (Stone et al., 1999).

The physical stress day included a maximal bicycle exercise tolerance test with radionuclide imaging. The physical stress data was not used because the current study investigated the relationship between mental stress and catecholamine/HRV. More details about these other study procedures may be found in work Kaufmann et al., (1998).

Speech task. During the mental stress speech task, patients were instructed to speak without interruption for 5 minutes on an assigned topic while being observed and supposedly evaluated by laboratory staff. The topic required difficult interpersonal role-playing. Patients had one minute to prepare a speech complaining to the staff of a nursing home about inadequate care given to a close relative. The staff played the role of the nursing home administration. After the speech task, patients were questioned for

presence or absence of stress-induced angina. If angina was present, its timing and duration were noted.

Data collection and measures

After a brief medical examination, a venous catheter was inserted. The patient received a light breakfast, and instruments were attached for recording a 12-lead electrocardiogram (ECG), ambulatory electrocardiogram (AECG), automated blood pressure recording, and drawing of blood. After a 30-minute rest period, blood was drawn for the determination of resting epinephrine and norepinephrine levels.

Heart rate variability. HRV was derived from AECG continuous monitoring during rest and stress task. The tapes were scanned at the HRV core laboratory at The Brigham and Women's Hospital on a Marquette Electronics system using standard procedures. Spectral analyses of digitized R-R intervals were conducted. The spectral HRV analyses method has been previously described in Rottman et al. (1990). Spectral analyses yield periodic and aperiodic frequency components of HRV. Periodic frequency domains are HF (0.15—0.4 Hz) in ms^2 and LF (0.04—0.15 Hz) in ms^2 . Aperiodic domains are ultra low frequency (ULF; 1.15×10^{-5} to 0.00335 Hz) in ms^2 and very low frequency (VLF; 0.0033 to 0.04 Hz) in ms^2 .

Blood pressure and HR. Blood pressure and heart rate were automatically recorded at baseline, 30 seconds after beginning the mental stress test and at one minute intervals after beginning the task.

Catecholamines. A blood sample for epinephrine and norepinephrine was drawn at baseline, every minute during mental stress task, and 10 minutes after completing the task. Blood samples were sent to the core laboratories for blinded analysis.

Data Analyses

SPSS version 12.01 software package from SPSS Inc. (2003) was used for the data analytical procedures.

Key Variables

Heart Rate Variability

Absolute units (HF and LF) and normalized units of HF and LF at baseline and during the speech task were used in data analysis. HF and LF were normalized (nHF and nLF) by dividing the power of individual periodic spectral components (HF and LF) by total HRV power, then subtracting the aperiodic components (ULF and VLF, e.g., < 0.01 Hz). This manipulation prevents very low frequency oscillations from masking other frequency components (HF and LF) (Aytemir et al., 2000). These normalized variables (nHF and nLF) were used for future descriptive and t-test analyses. HF and LF percentage variables were also created (HF/HRV total power and LF/HRV total power) done in order to control for the effects of HR on the changes in HF and LF. Each percentage variable was divided into 4 categories based on a quartile split and the quartiles were used in further multivariate analyses.

Catecholamines

Levels of hormones (Epi and NE) after 30 minutes of rest were used for baseline levels. Maximum levels of Epi and NE during the speech task were used for stress levels. In addition, the levels of Epi and NE at rest and during the speech task were categorized based on quartile split. Therefore, Epi and NE each had four levels from low to high. These new categories were used for MANOVAs and ANOVAs.

Hemodynamics

For the baseline measures of HR, SBP and DBP, the levels after 30 minutes of rest were used. Several BP measures were taken at baseline until BP was stable. For the stress levels, the maximum levels of HR, SBP and DBP during the speech task were used for the data analyses.

Statistical Analyses

Correlation analyses were performed to investigate relations among key variables (HRV, catecholamine levels, and hemodynamics). A series of paired t-tests were performed in order to find if there were any significant changes from baseline to stress in the variables of interest. For hypotheses I and II: a series of multivariate analyses of variance (MANOVA) were conducted to investigate the relations between HF (LF) and catecholamine levels at rest and during the speech task. To test these hypotheses, HRV (HF + LF) were analyzed as independent variables and catecholamine levels (Epi and NE) as dependent variables. For the sub-analyses, the change score from baseline to speech task was calculated for the epinephrine by subtracting baseline levels from

maximum levels during the speech task. For hypothesis III: a series of MANOVAs were conducted to investigate the relations between HF (LF) and hemodynamics (SBP, DBP, and HR) at rest and during the speech task. To test this hypothesis, HRV (HF + LF) were analyzed as independent variables and hemodynamics (HR, SBP, and DBP) as dependent variables.

A series of MANOVAs were conducted to investigate the relationships between epinephrine (norepinephrine) and hemodynamic variables (SBP, DBP, and HR) at baseline and during speech task. To test this hypothesis, catecholamine levels (Epi and NE) were analyzed as independent variables and hemodynamics (HR, SBP, and DBP) as dependent variables.

Significance levels were set at $\alpha = 0.05$ and used for all analyses unless otherwise noted.

Results

Sample Characteristics

Of the 196 PIMI participants, only 148 participants with available HRV data were used in the present study. The participants with stable CAD were between the ages of 40-70. The ages of the participants ranged as followed: 41-49 (9%), 50-59 (25.5%), 60-69 (47%), and 70-80 (18.1%). The sample consisted of 23 (15.4%) females, 125 (83.9 %) males; 121 (86.6 %) white, 18 (12.8 %) other; 100 (72.5%) married; 57

(40.2%) employed; and 108 (52%) had ≥ 12 years of education. Additional information about medical history and health habits are presented in Table 1.

Descriptive statistics

For all variables of interest, all scores ≥ 3 standard deviations above the mean on a variable were excluded as outliers from data analyses for that variable. The descriptive statistics for the HRV (nHF, nLF, and LF/HF) variables, catecholamine levels (Epi and NE) and hemodynamics (SBP, DBP, and HR) at baseline and during the speech task are presented in Table 2.

Manipulation check and correlations between variables

In order to insure that the mental stress procedure had an effect on the levels of the HRV, catecholamine and hemodynamics, a series of paired t tests were conducted (see Table 3). The paired t tests for HRV variables showed a marginally significant decrease in nHF from rest to stress, $t_{(142)} = 1.8, p = 0.07$, and no changes in nLF from rest to stress $t_{(147)} = .83, p = .41$. Catecholamine levels and hemodynamic variables showed significant increases from rest to stress ($p < 0.001$; see Table 2).

In order to determine the relationships between variables of interest at baseline and during the speech task, simple correlation analyses were performed. (See tables 4). The relationships between variables were more pronounced during speech than at baseline.

Hypothesis I

In addition to correlation coefficients, MANOVAs were performed with HF as an independent variable and Epi and NE as dependent variables in order to examine the

relationship between HF (4 levels, low to high) and catecholamine (Epi and NE) at baseline and during speech task.

I a. HF and Catecholamine relationship at rest

There were no significant relationships between nHF and catecholamine levels (Epi and NE) at rest ($r_{Epi} = .02$, $r_{NE} = .02$, $p > 0.10$).

Multivariate analyses of variance revealed no significant relationship between HF levels and catecholamine (Epi and NE), Multivariate $F_{(6, 266)} = .42$; $p = .87$ at baseline. Subsequent univariate analyses of variance (ANOVA) did not reveal any significant relationships between HF levels and catecholamine (Epi and NE) at baseline, $F_{Epi(3, 133)} = 0.44$; $p = 0.73$; $F_{NE(3, 133)} = 0.46$; $p = 0.71$. (See figures 2A and 2B.)

I b. HF and Catecholamine relationship during speech

During the speech task nHF was inversely related to catecholamine levels ($r_{Epi} = -.21$, $r_{NE} = -.19$; $p = .10$). MANOVA revealed a marginally significant relationship between HF levels and catecholamine (Epi and NE) during the speech task, Multivariate $F_{(6, 262)} = 1.8$; $p = .095$. See Figure 3. Univariate analyses of variance revealed a significant relationship between HF levels during the speech task and Epi, $F_{(3, 132)} = 2.9$; $p = .037$, and no significant relationship between HF levels during the speech task and NE $F_{(3, 132)} = 1.1$; $p = 0.34$, see Figure 3A and 3B. Dunnett's post hoc test demonstrated that the group with the lowest levels of HF during mental stress had the highest levels of Epi during speech in comparison to groups 2, $p = 0.02$; 3, $p = 0.02$; and group 4, $p = 0.04$. (See Figure 3A.)

To further investigate the relationship between epinephrine and HF during the speech task, changes in Epi from baseline to stress were examined in relation to levels of HF during the speech task using ANOVA. These analyses of variance revealed a significant relationship between HF levels during speech and epinephrine change scores, $F_{(3, 130)} = 2.9; p = .04$. Dunnett's post hoc test demonstrated that the group with the lowest levels of HF (group 1) had the highest increase in epinephrine from baseline to stress in comparison to groups 3, $p = 0.04$ and 4, $p = 0.03$. (See Figure 4.)

Hypothesis II

In addition to correlation coefficients, MANOVAs were performed with LF as an independent variable and Epi (NE) as a dependent variable in order to examine the relationship between LF (4 levels low to high) and catecholamine (Epi and NE) at baseline and during speech task.

II a. LF and catecholamine relationship at rest

There were no significant relationships between nLF and catecholamine (Epi and NE) at rest ($r_{Epi} = -.05$, $r_{NE} = -.04$, $p > .10$). Multivariate analyses of variance revealed no significant relationship between LF and catecholamine (Epi and NE), Multivariate $F_{(6, 260)} = .39; p = .88$. Univariate analyses of variance (ANOVA) did not reveal any relationships between LF levels and catecholamine (Epi or NE) at baseline, $F_{Epi (3, 131)} = 0.27; p = 0.85$; $F_{NE (3, 131)} = 0.64; p = 0.57$. (See Figure 5A and 5B.)

II b. LF and catecholamine relationship during speech

During the speech task nLF was inversely related to catecholamine levels ($r_{Epi} = -.30$,

$r_{NE} = -.23$; $p = .05$). MANOVA revealed a significant relationship between LF levels and catecholamine levels (Epi and NE) during speech, Multivariate $F_{(6, 258)} = 3.7$; $p = .001$. Univariate analyses of variance revealed a significant relation between LF levels during the speech task and Epi, $F_{(3, 130)} = 5.3$; $p = .002$. Dunnett's post hoc test demonstrated that the group with the lowest levels of LF (group 1) had highest levels of Epi during speech in comparison to groups 2, $p = .003$; 3, $p = .004$; and 4, $p = .001$. (See Figure 6A.) Univariate analyses of variance revealed a significant relation between LF levels during Speech and NE, $F_{(3, 130)} = 3.8$; $p = .012$. Dunnett's post hoc test demonstrated that the group with the lowest levels of LF had highest levels of NE in comparison to groups 2, $p = .06$; 3, $p = .005$; and 4, $p = .005$. (See Figure 6B.)

Hypothesis III

In addition to correlation coefficients a series of MANOVAs were conducted, using HF (LF) as an independent variable and hemodynamics (HR, SBP, and DBP) as dependent variables (IIIa and IIIb), in order to examine the relationships between HRV (quartile HF and LF levels) and hemodynamics (SBP, DBP, and HR) at baseline and during speech.

III a. Relationship between Hemodynamics and HRV (HF and LF) at rest

nLF was inversely related to systolic blood pressure at rest ($r = -.22$; $p = .05$). There were no other significant relationships among HRV variables (nHF and nLF) and hemodynamics at rest. See Table 3 for the correlation matrix for all the key variables at baseline.

HF and Hemodynamics. The MANOVA revealed no significant relationship between HF and hemodynamics (SBP, DBP, and HR) at baseline, Multivariate $F_{(9, 341)} = 0.74$; $p = 0.68$. Univariate analyses of variance also did not reveal any relationship between HF levels and hemodynamics (SBP, DBP, and HR) at baseline, $F_{SBP (3, 142)} = 0.47$; $p = 0.70$; $F_{DBP (3, 142)} = 1.31$; $p = 0.27$; and $F_{HR (3, 142)} = 0.13$; $p = 0.94$.

LF and Hemodynamics. The MANOVA revealed no significant relationship between LF and hemodynamics (SBP, DBP, and HR) at baseline, Multivariate $F_{(9, 334)} = 1.14$; $p = 0.34$. Univariate analyses of variance did not reveal any relationships between LF levels and hemodynamics (SBP, DBP, and HR) at baseline, $F_{SBP (3, 139)} = 1.18$; $p = 0.32$; $F_{DBP (3, 139)} = 0.49$; $p = 0.67$; and $F_{HR (3, 139)} = 0.71$; $p = 0.55$.

III.b. Relationship between Hemodynamics and HRV (HF and LF) during Speech

There were inverse relationships among HRV (nLF and nHF) variables and SBP with HR (correlations ranged from $r = -0.42$ to -0.20 with p level between 0.05 and 0.10) during the speech task. There were no significant relationships among HRV (nHF and nLF) variables and DBP. (See tables 3 and 4.)

HF and Hemodynamics. Multivariate analyses of variance revealed a significant relationship between HF and hemodynamics (SBP, DBP, and HR) during speech, Multivariate $F_{(9, 338)} = 2.8$; $p = 0.03$. Univariate analyses of variance revealed significant relationships between HF levels and SBP and HR during Speech, $F_{SBP (3, 141)} = 3.9$; $p = 0.01$; and $F_{HR (3, 141)} = 7.2$; $p < 0.001$. The relationship between HF levels and DBP

during speech was not significant, $F_{DBP(3, 141)} = 1.14$; $p = 3.4$. Dunnett's post hoc test demonstrated that the group with the lowest levels of HF (group 1) had the highest levels of SBP and HR during speech in comparison to groups 3, $p_{SBP} = 0.04$, $p_{HR} = 0.001$; and 4, $p_{SBP} = 0.001$, $p_{HR} < 0.001$. See Figures 7A and 7B.

LF and Hemodynamics. Multivariate analyses of variance revealed a significant relationship between LF and hemodynamics (SBP, DBP, and HR) during Speech, Multivariate $F(9, 336) = 4.1$; $p < 0.001$. Univariate analyses of variance revealed significant relationships between LF levels and SBP and HR during Speech, $F_{SBP(3, 140)} = 8.9$; $p < 0.001$; and $F_{HR(3, 140)} = 4.0$; $p = 0.009$. The relationship between LF levels and DBP during Speech was marginally significant, $F_{DBP(3, 140)} = 2.5$; $p = 0.06$. Dunnett's post hoc test demonstrated that the group with the lowest levels of LF (group 1) had highest levels of SBP and HR during Speech in comparison to groups 2, $p_{SBP} = 0.02$, $p_{HR} = 0.02$; 3, $p_{SBP} = 0.01$, $p_{HR} = 0.04$; and 4, $p_{SBP} < 0.001$, $p_{HR} = 0.01$. (See Figures 7A and 7B.)

Relationship between Hemodynamics and Catecholamines (Epi and NE)

In addition to correlation coefficients, a series of MANOVAs were conducted, using Epi (NE) as an independent variable and hemodynamics (HR, SBP, and DBP) as dependent variables, in order to examine the relationships between catecholamine (quartile Epi and NE levels) and hemodynamics (SBP, DBP, and HR) at baseline and during speech.

At rest

Diastolic blood pressure positively related to NE at rest ($r = .19$; $p < .01$). There were no other significant relationships between catecholamine levels and hemodynamics at baseline. (See table 3.)

Epinephrine and Hemodynamics. The MANOVA revealed a significant relationship between Epi and hemodynamics (SBP, DBP, and HR) at baseline, Multivariate $F_{(9, 324)} = 2.2$; $p = .02$. Univariate analyses of variance revealed only one significant relationship: DBP and Epi were related at baseline, $F_{DBP (3, 135)} = 3.5$, $p = 0.02$.

Norepinephrine and Hemodynamics. The MANOVA revealed no significant relationships between NE and hemodynamics (SBP, DBP, and HR) at baseline, Multivariate $F_{(9, 324)} = 1.5$; $p = 1.6$.

During Speech

There were significant and marginally significant positive relationships between catecholamine level (Epi and NE) and hemodynamics (SBP, DBP, and HR) during the speech task (Pearson correlations ranged from $r = .20$ to $.44$; $p = .05-.10$). See table 4 for the correlation matrix.

Epinephrine and Hemodynamics. The MANOVA revealed a significant relationship between Epi and hemodynamics (SBP, DBP, and HR) during the speech task, Multivariate $F_{(9, 323)} = 3.6$; $p < 0.001$. Univariate analyses of variance revealed significant relationships between Epi levels and SBP and HR during Speech, $F_{SBP (3, 135)} = 3.7$; $p = 0.03$; and $F_{HR (3, 135)} = 7.8$; $p < 0.001$. The relationship between Epi levels and DBP during Speech was not significant, $F_{DBP (3, 135)} = 2.0$; $p = 0.11$. Dunnett's post hoc test demonstrated that the group with the lowest levels of Epi (group 1) had the lowest

levels of SBP and HR during Speech in comparison to groups 3, $p_{HR} = 0.01$; and 4, $p_{SBP} = 0.04$, $p_{HR} < 0.001$.

Norepinephrine and Hemodynamics. The MANOVA revealed significant relationships between NE and hemodynamics (SBP, DBP, and HR) during the speech task, Multivariate $F_{(9, 331)} = 1.95$; $p = 0.05$. Univariate analyses of variance revealed significant relationships between NE levels and SBP and DBP during Speech, $F_{SBP (3, 138)} = 3.2$; $p = 0.02$; and $F_{DBP (3, 138)} = 3.6$; $p = 0.15$. The relationship between NE levels and HR during Speech was not significant, $F_{HR (3, 138)} = 2.1$; $p = 0.11$.

Discussion

The primary aim of this study was to explore the relationships among various markers of sympatho-vagal balance of the ANS (HF HRV, LF HRV, Epi, NorEpi, HR, and BP) in CHD patients at rest and during acute mental stress. As predicted, the results of the present study showed a significant decrease in HF HRV levels from rest to stress. While there were no significant relationships among HF, catecholamine level, and hemodynamics at rest, there were significant relationships present during stress. Furthermore, the lowest levels of HF corresponded to highest levels of Epi, SBP, and HR during mental stress.

HF changes from rest to stress

As expected, there was a marginally significant decrease in nHF from rest to stress. These results coincide with previous findings (Hjortskov et al., 2004; Ruediger et al., 2004; Lampert, et al., 2005; Lane et al., 1992). It is believed that HF reflects the

vagal control of S-A node, one important aspect of the parasympathetic control of ANS of the heart (Lombardi et al., 1996). At rest, the autonomic nervous stimulation of the heart is predominantly represented by parasympathetic influences (Bernardi et al., 1989). However, during mental stress, because of the increased demand on the heart muscle, the balance shifts towards sympathetic influences due to vagal withdrawal or/and increase of sympathetic stimulation (Furlan et al., 1990; Pagani et al., 1991). Therefore, during mental stress the subsequent decrease in HF was expected and confirmed.

There were no differences in LF (measured in normal units) levels from rest to stress. These results coincide with the research by Hjortskov et al., (2003) that evaluated changes in HRV components from rest to computer-related mental stress in 12 healthy participants. Despite a significant decrease in HF, LF remained unchanged from rest to stress (Hjortskov et al., 2004). In contrast, other researchers have found a significant increase in levels of LF from rest to stress (Bernardi et al., 2000; Miyake, 1997; Pagani, Mazzuero et al., 1991). These discrepancies with previous research may be attributable to the length of the psychological stressor. Some studies (Bernardi et al., 2000; Miyake 1997; Pagani 1991) used a prolonged psychological stressor. According to Stein and colleagues (2005) and Kleiger (2005), immediate fluctuations of HRV are modulated by parasympathetic nervous system (RSA); the slower fluctuations of HRV are modulated by sympathetic nervous system, through activation of the baroreflex. In other words, to see changes in LF, more time may be needed to demonstrate the effects of sympathetic nervous system on the SA.

Hypothesis I

The hypothesis that there will be no relationship between the levels of HF and catecholamine at rest; and that CHD patients with lower levels of HF will have higher levels of catecholamine during mental stress, was partially confirmed by the present results. In the present study, there was no relationship between HF levels and catecholamine at rest. However, during mental stress, patients with lowest levels of HF had the highest levels of epinephrine. Furthermore, patients with the highest increase in epinephrine levels from rest to mental stress had the lowest levels of HF during mental stress. Epinephrine and norepinephrine are neurohumoral markers of the sympathetic nervous system (Hirayanagi, et al., 2003). The increases in plasma levels of norepinephrine and epinephrine reflect an activation of the sympathetic branch of ANS. Some of the organs and systems are under the control of both sympathetic and parasympathetic ANS. Activation of one of the branches of ANS is followed by inactivation of another branch (Ingemansson, et al., 1998). In the normal heart, vagal tone is dominant during rest (Bernardi et al., 2000; Miyake, 1997; Pagani, Mazzuero et al., 1991; Takei et al., 1992; Takei et al., 1991). However during mental stress, the balance shifts towards the sympathetic system (Soufer, 2004; McEwen, 2004). In addition, it is believed that in CHD patients the overall sympatho-vagal balance is shifted towards the sympathetic nervous system (Joho et al., 1999; Lanza et al., 1996). This dysregulation is especially pronounced during mental stress (Lumpert et al., 2004; Ramachndruni et al., 2006). HF represents parasympathetic control of the heart, and plasma levels of epinephrine represent sympathetic activity. Therefore, it was expected that HF and epinephrine should be inversely related during mental stress in CHD patients. In the present study, patients with lowest level of HF (higher levels of vagal

withdrawal) had the highest increases in epinephrine (higher activation of sympathetic nervous system).

In addition, a body of research has shown that patients, who have low levels of HF during rest and especially during mental stress, are at higher risk of subsequent cardiac events (e.g., MI, SCD) (Gianaros et al., 2005; Ozdemir et al., 2003). This relationship is largely attributed to a decrease of the parasympathetic tone on the heart and an increase in sympathetic tone, which, in turn, leads to ventricular arrhythmias (Kop et al., 2001; Ruediger et al., 2004). Furthermore, high levels of plasma epinephrine in CHD patients have been predictive of future cardiac arrhythmias through beta-adrenergic receptor stimulation (Flack & Yunis, 1997). Therefore, the use of these two markers of autonomic functioning (HF and epinephrine), instead of using only catecholamine levels or HRV may give more information about the stage of the sympatho-vagal balance of patient and help to identify the patients at higher risk of future cardiac adverse events earlier.

There was no relationship between HF and norepinephrine during mental stress. These findings coincide with previous research. Kurita et al., (1999) investigated the relationship between HF and circulating norepinephrine during handgrip exercises in cardiac patients (n=20) and normal controls (n =12). In normal subjects a significant inverse relationship was obtained between HF and norepinephrine, however there was no relationship between HF and norepinephrine in CAD patients. Researchers did not assess the levels of epinephrine in the study.

Epinephrine and norepinephrine are secreted by the adrenal medulla as part of the overall sympathetic activation of the body in response to mental stress (Howley,

1976; Mason, 1968a). However plasma epinephrine and norepinephrine are indirect markers of the sympathetic branch of the ANS. In addition, due to the short life of these hormones (1-3 minutes) (Hirayanagi, Nakabayashi, Okonogi, & Ohiwa, 2003) and their reuptake by the sympathetic receptors of various organs including the heart and blood vessels, venous blood contains less catecholamine than was originally released by the adrenal gland. Time and type of blood (venous versus arterial) are very important factors due to rapid effects of the sympathetic nervous system. Norepinephrine released by cardiac nerves is rapidly taken up by the heart and does not contribute to plasma catecholamines in healthy subjects. In subjects with chronically increased sympathetic stimulation, i.e. CHF patients, the uptake mechanisms may become overwhelmed, resulting in “spillover” into the coronary sinus effluent (reference). Therefore, interpretation of the relationship between catecholamine and HF must be undertaken carefully. The previously noted factors such as venous blood, short-half life of NE, and severity of cardiac disease may influence the lack of the relationship between HF and norepinephrine during mental stress in present study.

Hypothesis II

Hypothesis II was not supported. The highest levels of LF were predicted to correspond to the highest levels of catecholamine. However, the results showed that the highest levels of LF were associated with the lowest levels of Epi and NorEpi during mental stress. This discrepancy can be explained by taking into consideration the length of the psychological stressor and vagal withdrawal. The LF component of HRV is thought by most investigators to represent both sympathetic and parasympathetic

modulations of ANS on S-N (Stein & Reddy, 2005). The rapid fluctuations of HRV usually reflect the parasympathetic control of the heart, and the slower fluctuations of HRV reflect combined sympathetic and parasympathetic control of the heart (Kamath et al., 1987). Therefore, the changes in HRV due to the sympathetic influences take longer to occur. During a short period of mental stress, as in the present study, LF changes may only reflect the parasympathetic influences. Kop et al. (2001) and Mussalo et al., (2003) found a decrease in LF during short mental activities (Kop et al., 2001; Mussalo, Vanninen, Ikaheimo, Laitinen, & Hartikainen, 2003). It is possible that, in the present study, the short duration of the psychological stressor of 5 minutes may have resulted in the sympathetic portion of LF remaining unchanged. LF in the present study may predominately reflect parasympathetic influences. If this were true, lower levels of LF would be expected to be inversely related to the levels of catecholamine (sympathetic markers), as well as SBP and HR (predominantly regulated by sympathetic nervous system), which is confirmed by the present study.

Although some researchers purport that the LF component of HRV in normalized units is a marker of sympathetic modulation of the S-A node and the evaluation of the LF/HF can assess the state of the sympatho-vagal balance (Malliani, et al., 1994), factors such as duration of the stressor and vagal withdrawal may play an important role in evaluation of LF and LF/HF at rest and during mental stress as markers of the sympathetic modulation on the S-A node, as shown in the present study.

Hypothesis III

Hypothesis III (a) at rest there will be no relationships between HF (LF) and SBP and DBP and HR; and (b) during mental stress, HF will have an inverse relation to hemodynamics, and LF will have positive relation with hemodynamics, was partially confirmed. The present study showed no relationship among HF and hemodynamics at rest. However, under mental stress conditions, patients with the lowest levels of HF had the highest levels of SBP and HR. There was no relationship between DBP and HF during speech. These findings coincide with previous findings (Lane, 1992; Kurita, et al., 1999).

The control of BP is largely under regulation of the sympathetic nervous system (Guyton & Hall, 2001; Mohrman, 1977). Activation of the sympathetic branch of the ANS is followed by a subsequent increase in blood pressure and a decrease in parasympathetic stimulation, represented by decrease in HF. Therefore, use of the blood pressure in relation to HF levels during stress allows a more comprehensive evaluation of the sympatho-vagal balance by providing information about sympathetic and parasympathetic branches of ANS. Therefore it may give a better understanding about the current stage of the autonomic balance of the individual and help to identify the patients at higher risk of future cardiac aversive events at earlier stages in their disease progression.

As predicted, there were no relationships between LF and hemodynamics at rest. However, during mental stress, patients with lower levels of LF had higher levels of SBP and HR, contrary to the original hypothesis. These findings can be explained similarly to the findings for the hypothesis II. The LF component of HRV is thought to represent both sympathetic and parasympathetic modulations of ANS on SA node, due

to short duration of the mental stress the changes in LF were mostly represented by parasympathetic influences.

Study limitations

The present study sample predominantly consisted of male Caucasian CHD patients. Therefore, the findings of this study may be limited only to this population. According to Kaufmann and colleagues (1998), because consent of the attending physician was required in order to discontinue cardiac medication, there is a possibility that the sample may also be biased towards less sick patients.

The activation of the sympathetic system during mental stress leads to an increase in heart rate. This increase in the heart rate is reflected in the subsequent decrease of HF power and an increase in LF power. In order to eliminate the effects of HR on HF (LF) and measure the true state of sympatho-vagal balance of heart regulation, percentages of HF (LF) were calculated from total HRV power. In addition, in order to investigate the relationships among HRV components (HF and LF) and catecholamine levels and hemodynamics, HF and LF percentages of HRV total power at rest and during stress were categorized based on a quartile split. Therefore, HF (LF) at rest was represented by four groups or levels (low to high), and HF (LF) during stress was represented by four levels (low to high). The use of the quartile split for HF (LF) based on percentage of HF (LF) from total HRV did not allow for the investigation of relationships among HF (LF), catecholamine, and hemodynamics. The same subject may have belonged to a different category of the HF (LF) at rest versus during stress.

In the present study, the mental stressor lasted only 5 minutes. The length of the stressor may not have been sufficient to show any significant changes in LF due to sympathetic activation. Therefore, it is important in the future study to use a longer duration of the mental stressor to investigate the relationship among LF power, catecholamine and hemodynamics.

Future implications

In order to further examine the clinical predictive value of this range of autonomic markers and to enable early identification of the individuals at the higher risk of subsequent coronary events, prospective case-control studies of CHD patients are needed. Case-control studies will allow researchers to determine if patients with higher levels of the sympatho-vagal imbalance assessed by the range of proposed markers have higher levels of adverse cardiac events during follow-up in comparison to patients with lower levels of the sympatho-vagal imbalance.

The role of the autonomic nervous system is to regulate and coordinate activities to ensure homeostasis so that humans can cope with ever-changing demands in daily life. An imbalance in this system, especially decreased parasympathetic activity and/or increased sympathetic activity in the regulation of the heart, has been linked to an increase of cardiac mortality in CHD patients. Changes in various markers of the sympatho-vagal balance (e.g., decreased HF, BP, and increases in catecholamine) alone may not be sufficient to risk stratify patients in order to predict the future adverse cardiac events. Sympatho-vagal imbalance may be due to vagal withdrawal and/or sympathetic over activity. The use of the multiple markers may improve the risk stratification by giving more comprehensive information about parasympathetic and

sympathetic influences on the heart. Clinicians may be able to use the additional information gained to prevent the negative cardiac outcomes. Therefore, it would be important to continue this line of research in order to fully understand the complex interplay between HR, HRV, BP and catecholamine levels.

Some evidence indicates that individuals with exaggerated cardiovascular changes in response to real-life or laboratory-induced psychological challenges are at higher vulnerability for CHD. However, relationships among HRV components, catecholamine and hemodynamics in terms of stress reactivity (change-scores) have not yet been investigated. Investigation of the relations of the change score of the proposed markers will expand the current knowledge about stress reactivity and vulnerability to future CHD.

In order to further investigate the relationships and prognostic value of the range of various markers of sympatho-vagal balance, it would be important to study the relationships among other proposed markers of the current study and baroreflex sensitivity. Impulses generated in the baroreceptors inhibit the tonic discharge of the vasoconstrictor nerves and excite the vagal innervations of the heart (Spallone & Menzinger, 1997). This, in return, causes vasodilation, and decrease in BP, HR and cardiac output (Spallone & Menzinger, 1997). The decreased sensitivity of the baroreflex has been related to increased mortality from MIs and arrhythmias in CHD patients (La Rovere, Gnemmi, & Vaccarini, 2001; La Rovere, Pinna et al., 2001). This relationship has been attributed to the presence of sympatho-vagal imbalance in the regulation of the baroreflex (e.g. sympathetic over activity) (La Rovere, et al., 2001). In the current study, BP was measured every minute. In order to investigate baroreflex

sensitivity the BP has to be measured beat to beat. The study of baroreflex sensitivity and its relation to components of HRV, catecholamine levels and hemodynamics would be an important addition to the body of research focusing on various markers of the future adverse cardiac events.

Finally, the nature of LF remains controversial. LF power is modulated by baroreflexes and a combination of sympathetic and parasympathetic efferent impulses on the SA node (Billman et al., 1990; Bloomfield et al., 1997). However, some research indicates that LF and changes in LF predominantly represent the sympathetic regulation or sympathetic activation of the heart (Princi et al., 2006; Piccirillo et al., 2006; Montano et al., 1996; Malliani et al., 1991; Pagani et al., 1991). Even though various mathematical manipulations of LF power have been used to better assess the sympathetic control of the heart (e.g. normalization of LF power, use of the ratio LF/HF), these manipulations of LF power have produced mixed results. It is believed that in the present study LF reflects mostly the parasympathetic modulation of heart rate variability due to the short duration of the stressor. Continued investigation into the nature of LF under varying stress conditions of differing lengths will be essential to determine under what conditions LF reflects sympathetic (parasympathetic) modulation of cardiac control.

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Appendix A: Tables

Table 1

Medical History and Health Habits (n = 147)

Characteristics	Frequency (%)
Current smoker	25 (16.8%)
Coronary angioplasty	69 (35.2%)
History of myocardial infarction	57 (38.3%)
History of congestive heart failure	5 (3.4%)
History of diabetes	20 (13.4%)
History of hypertension	67 (45%)

Table 2

Means and Standard Deviations of Measures During Baseline and Stress

Measures	Baseline		Stress	
	Mean	<i>SD</i>	Mean	<i>SD</i>
Normalized HF Power	6.5	5.3	5.4	4.0
Normalized LF Power	21.2	12.2	22.1	13.8
Norepinephrine	394	167	527	218
Epinephrine	27.1	21.2	60.4	39.4
Systolic Blood Pressure	144	19	187	26.3
Diastolic Blood Pressure	78.7	10.2	98.7	14.5
Heart Rate	70.7	10.8	84.4	14.7
LF to HF Ratio	4.6	3.4	5	4.1

Table 3

Paired t – tests Comparing Measures Taken During Baseline versus During Stress

Measures	t	df	p (two-tailed)
Normalized HF Power	1.8	142	0.07
Normalized LF Power	0.83	147	0.41
Norepinephrine	10.6	141	<0.001
Epinephrine	12.1	136	<0.001
Systolic Blood Pressure	28.9	148	<0.001
Diastolic Blood Pressure	24.7	148	<0.001
Heart Rate	16.5	148	<0.001
LF to HF ratio	1.2	145	0.23

Table 4

Intercorrelations among HRV Components, Catecholamine Levels and Hemodynamics at Baseline and during Stress

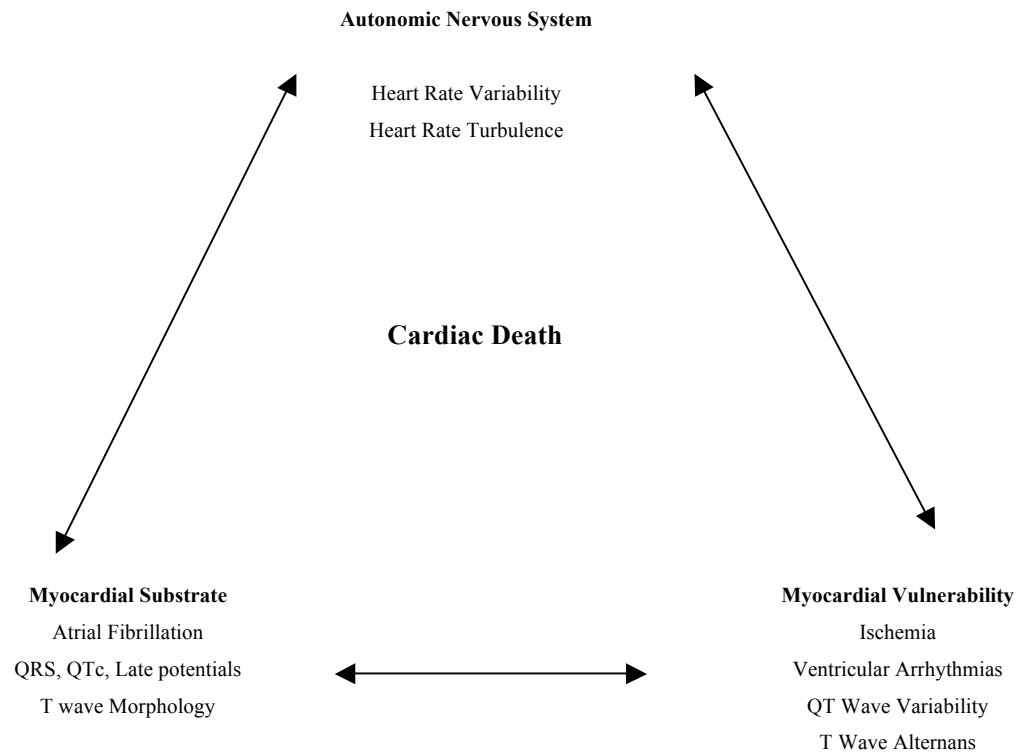
Variables	nHF	nLF	LF/HF	Epi	NE	SBP	DBP	HR
At Baseline ($n = 147-140$)								
nHF		.27**	-.52**	.02	.02	-.02	-.16*	.00
nLF			.38**	-.05	-.04	-.22**	.01	.05
LF/HF				.02	-.02	-.13	.14	.16
Epi					.30**	.07	.15	.10
NE						.14	.19*	.05
SBP							.51**	.14
DBP								.21**
HR								
During Speech ($n = 147-140$)								
nHF		.43**	-.35**	-.21*	.03	-.20*	-.13	-.34**
nLF			.36**	-.30**	-.23**	-.42**	-.14	-.29**
LF/HF				-.13	-.13	-.18*	-.08	.08
Epi					.24**	.36**	.19*	.36**
NE						.28**	.22**	.20*
SBP							.70**	.44**
DBP								.29**
HR								

* $p < 0.10$, ** $p < 0.05$, two-tailed. nHF = Normalized HF, nLF = Normalized LF, Epi = Epinephrine, NE = Norepinephrine, SBP = systolic blood pressure, DBP = diastolic blood pressure, and HR = heart rate

Appendix B: Figures

Figure 1

Factors contributing to cardiac death



Adopted from Zareba et al., 2003

Figure 2

Study design

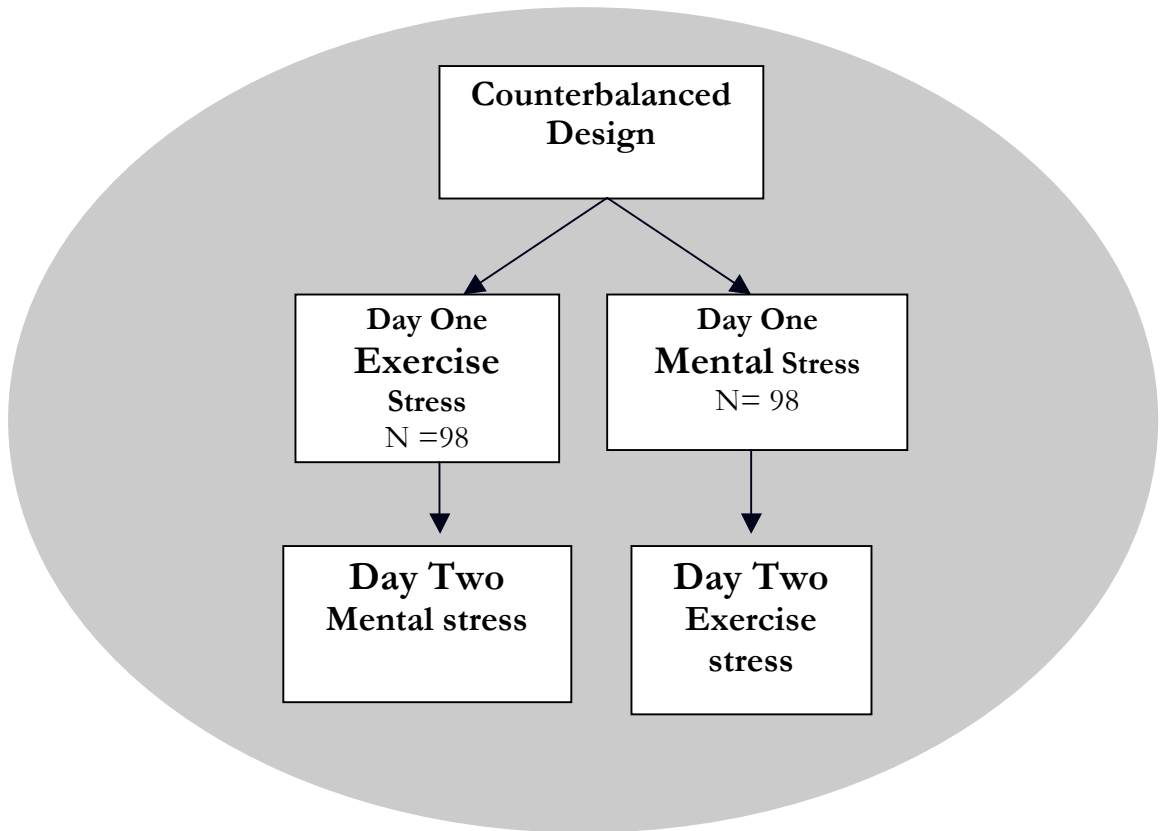
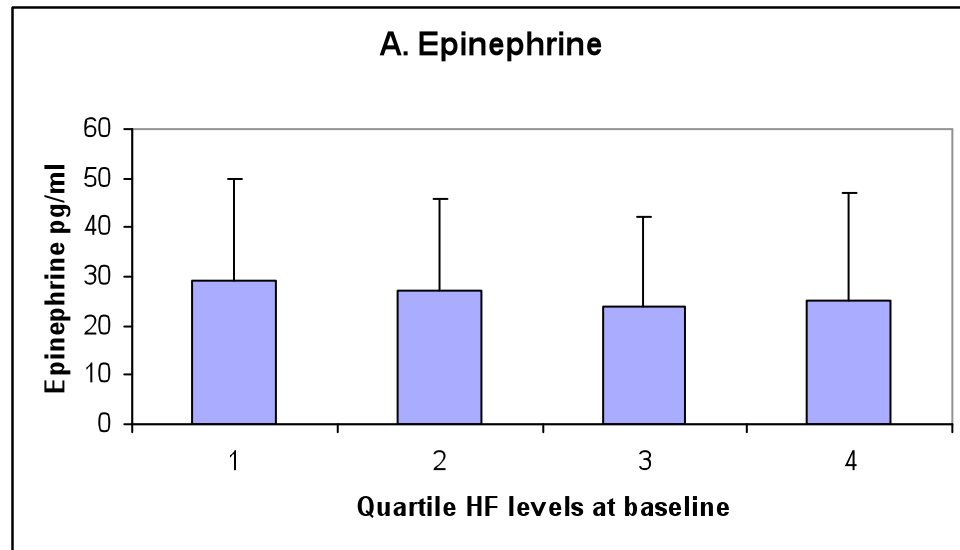


Figure 3

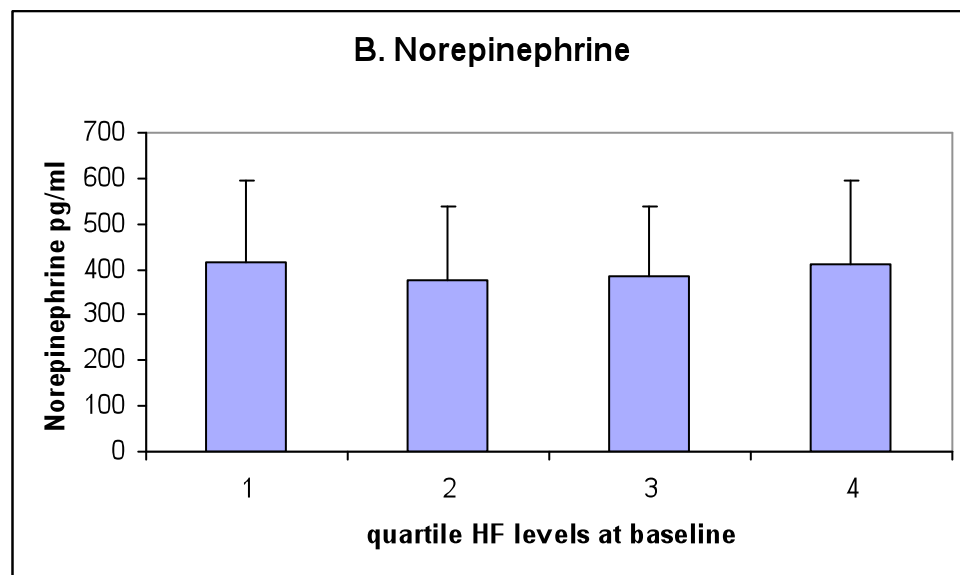
Relationship between catecholamine levels ($M \pm SD$)

A. Epinephrine; B. Norepinephrine and HF at baseline.

HF was divided into quartiles, with 1 = lowest and 4 = highest quartile.

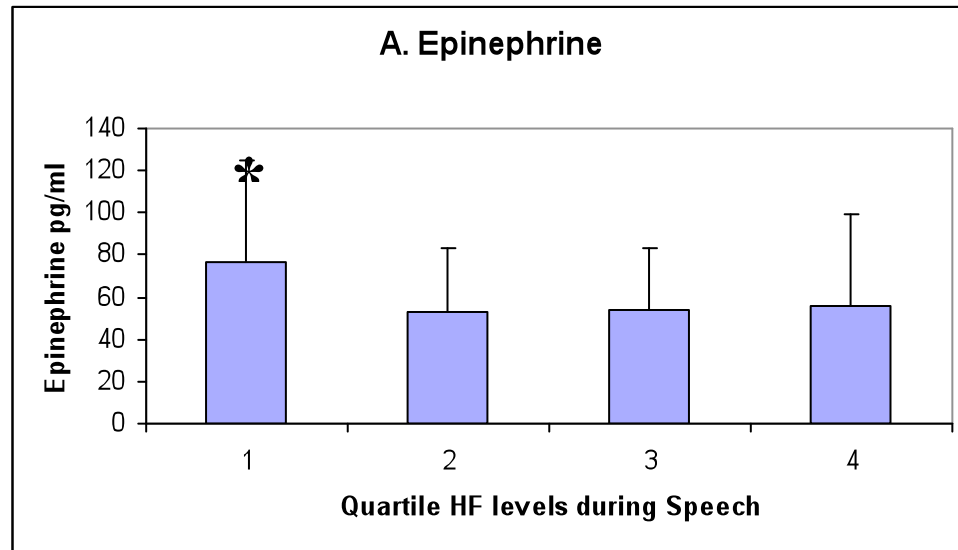


$$F_{Epi(3, 133)} = 0.44; p = 0.73$$



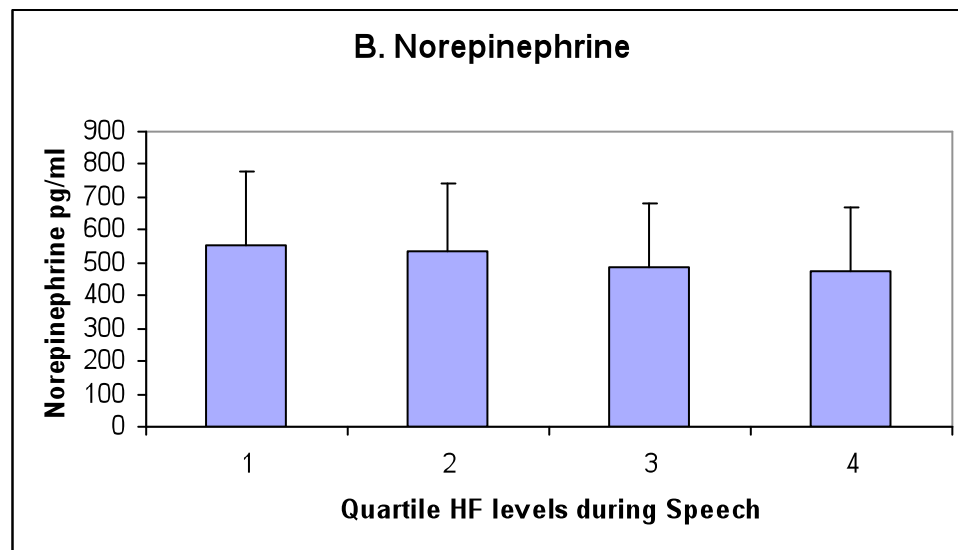
$$F_{NE(3, 133)} = 0.46; p = 0.71$$

Figure 4
 Relationship between catecholamine levels ($M \pm SD$)
 A. Epinephrine; B. Norepinephrine and HF during speech.
 HF was divided into quartiles, with 1 = lowest and 4 = highest quartile.



$$F_{\text{EPI}}(3, 132) = 2.9; p = .037$$

* Group 1 (lowest levels of HF) had the highest levels of Epi in comparison to Group 2, $p = 0.02$; Group 3, $p = 0.02$; and Group 4, $p = 0.04$.

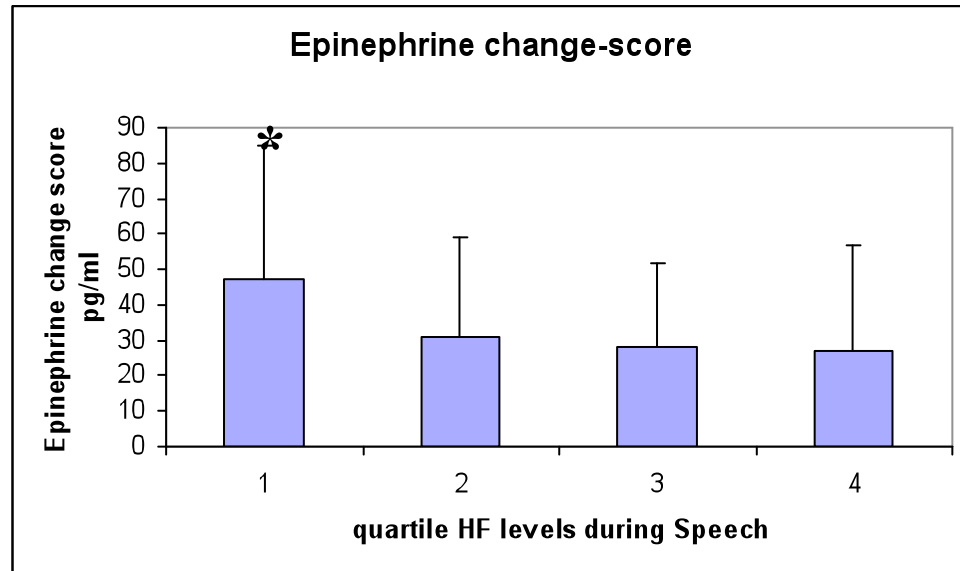


$$F_{\text{NE}}(3, 132) = 1.1; p = 0.34$$

Figure 5

Epinephrine change-score ($M \pm SD$) by HF during speech:

HF (speech) was divided into quartiles, with 1 = lowest and 4 = highest.



$$F_{(3, 130)} = 2.9; p = .04$$

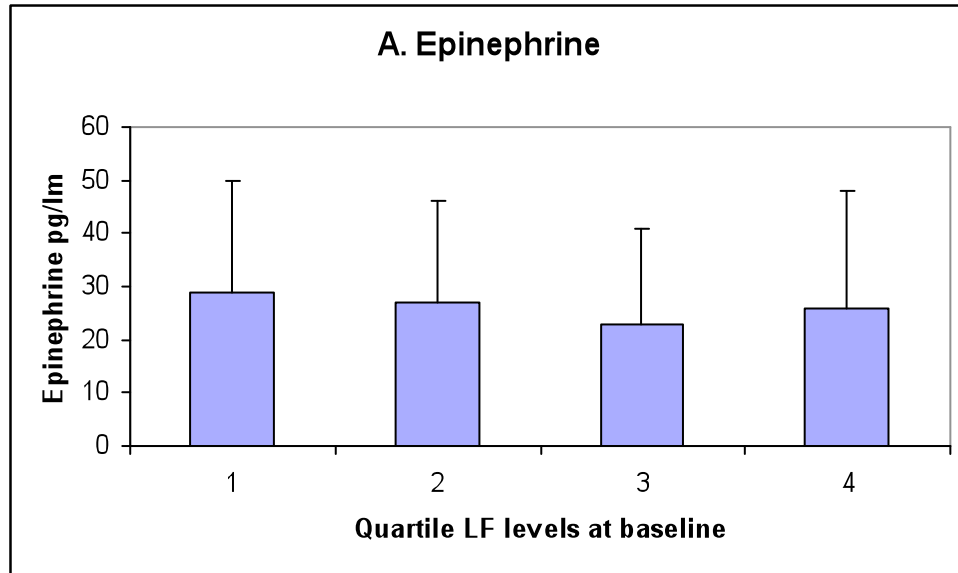
* Group 1 (lowest levels of HF) had highest increase in epinephrine from baseline to stress in comparison to Group 3, $p = 0.04$ and Group 4, $p = 0.03$.

Figure 6

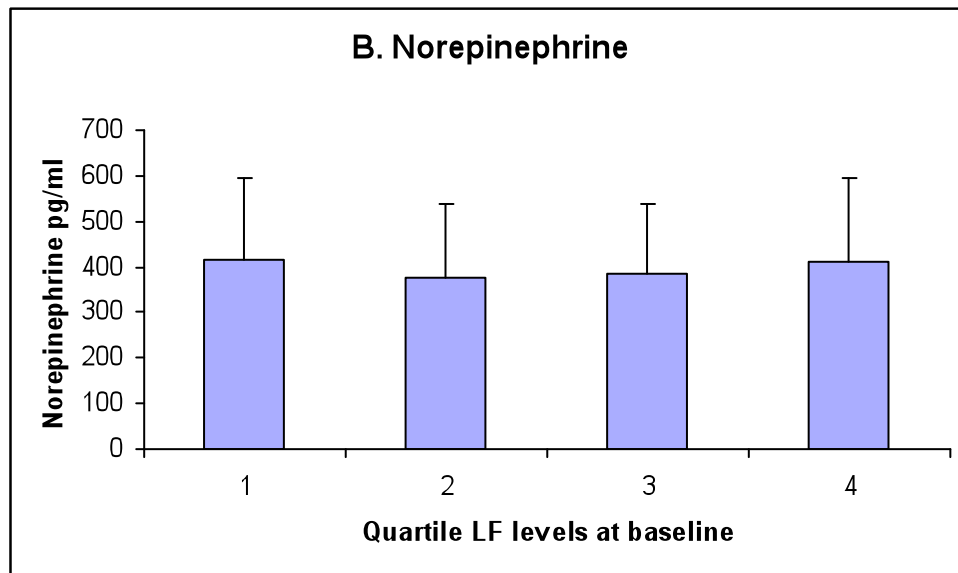
Relationship between catecholamine levels ($M \pm SD$)

A. Epinephrine; B. Norepinephrine and LF at baseline.

LF was divided into quartiles, with 1 = lowest and 4 = highest quartile.

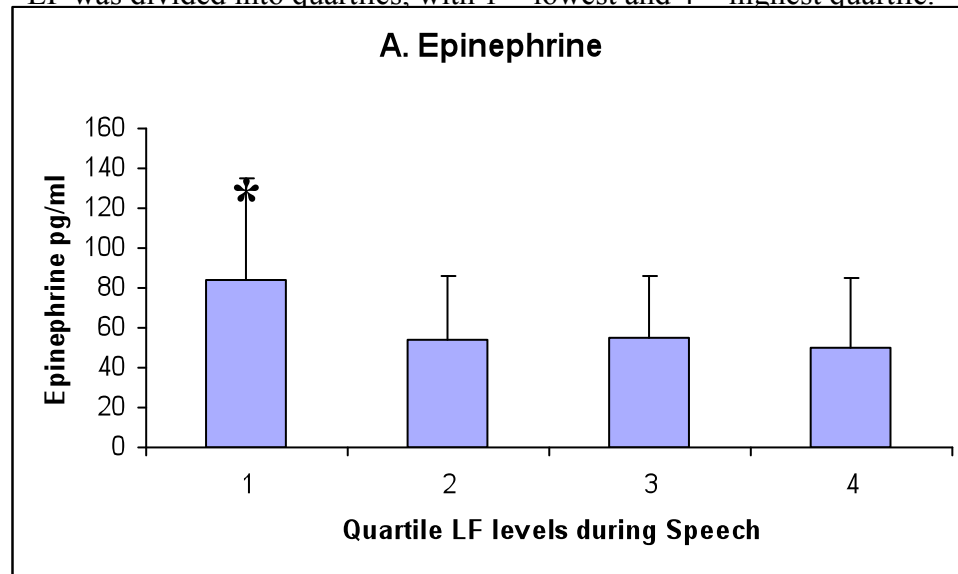


$$F_{Epi(3, 131)} = 0.27; p = 0.85$$



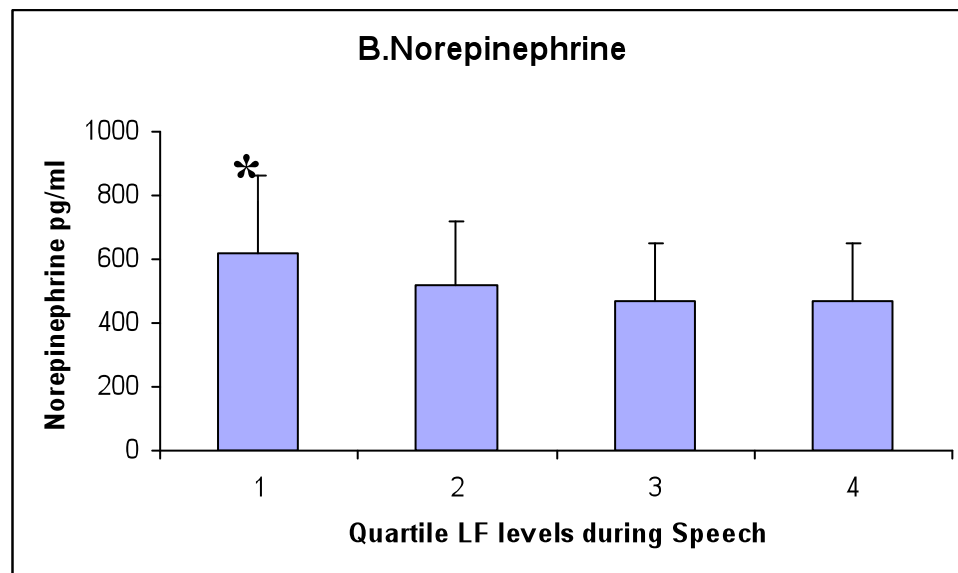
$$F_{NE(3, 131)} = 0.64; p = 0.57$$

Figure 7
 Relationship between catecholamine levels ($M \pm SD$)
 A. Epinephrine; B. Norepinephrine and LF during speech.
 LF was divided into quartiles, with 1 = lowest and 4 = highest quartile.



$$F_{\text{Epi}}(3, 130) = 5.3; p = 0.002;$$

* Group 1 (lowest levels of LF) had highest levels of Epi in comparison to Group 2, $p = .003$; Group 3, $p = .004$; and Group 4, $p = .001$.



$$F_{\text{NE}}(3, 130) = 3.8; p = 0.01$$

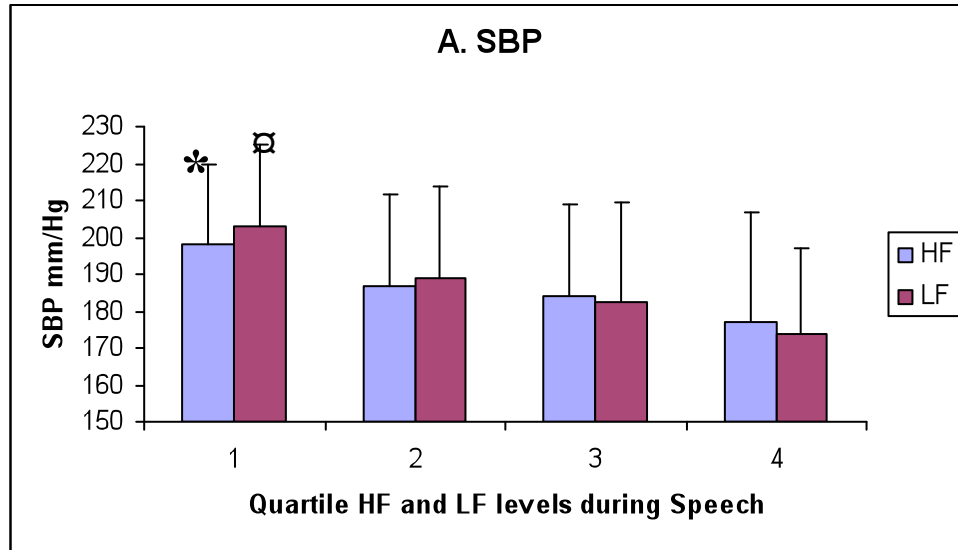
*Group 1 (lowest levels of LF) had highest levels of NE in comparison to Groups 2, $p = .06$; Group 3, $p = .005$; and Group 4, $p = .005$.

Figure 8

Relationship between Hemodynamics ($M \pm SD$)

A. Systolic BP; B. Heart rate and HRV (HF and LF) during speech.

HF (LF) was divided into quartiles, with 1 = lowest and 4 = highest quartile.

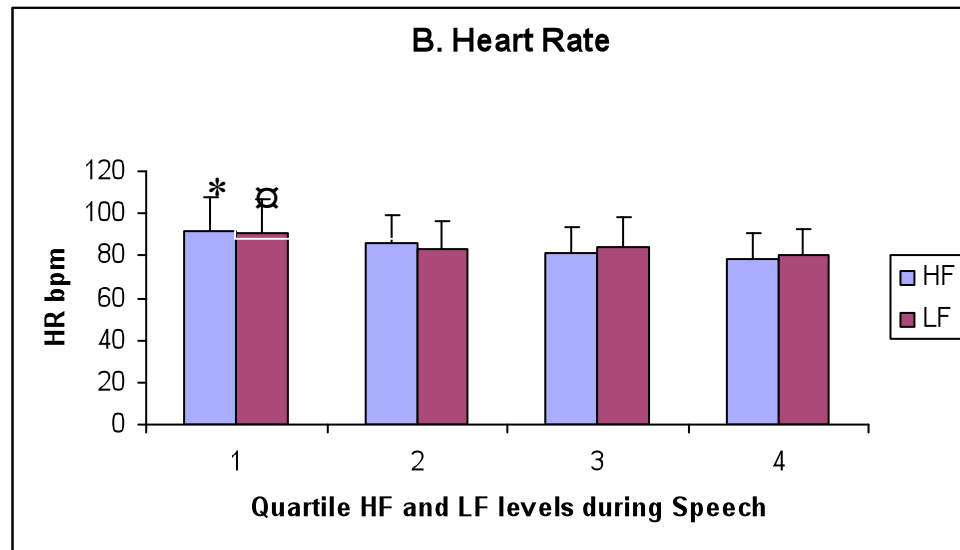


$$F_{HF} (3, 141) = 3.9; p = 0.01$$

* Group 1 (lowest levels of HF) had highest levels of SBP in comparison to Group 3, $p = 0.04$ and Group 4, $p = 0.001$.

$$F_{LF} (3, 140) = 8.9; p < 0.001$$

□ Group 1 (lowest levels of LF) had highest levels of SBP in comparison to Group 2, $p = 0.02$; Group 3, $p = 0.01$; and Group 4, $p_{SBP} < 0.001$.



$$F_{HF(3, 140)} = 4.0; p = 0.009.$$

* Group 1 (lowest levels of HF) had highest levels of HR in comparison to Group 3, $p = 0.001$ and Group 4, $p < 0.001$.

$$F_{LF(3, 141)} = 7.2; p < 0.001.$$

□ Group 1 (lowest levels of LF) had highest levels of HR in comparison to Group 2, $p = 0.02$; Group 3, $p = 0.04$; and Group 4, $p = 0.01$.